Postnatal maturation of vagal respiratory reflexes in preterm and full-term lambs

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Arsenault, Julie, François Moreau-Bussière, Philippe Reix, Théophile Niyonsenga, and Jean-Paul Praud. Postnatal maturation of vagal respiratory reflexes in preterm and full-term lambs. J Appl Physiol 94: 1978–1986, 2003. —The postnatal development of ventilatory reflexes originating from bronchopulmonary receptors was assessed in preterm vs. full-term lambs. Ventilation and arterial pressure were repeatedly measured in 10 preterm (gestational age, 132 days) and 7 full-term lambs without sedation from day 1 to day 42. The Hering-Breuer inhibitory reflex (slowly adapting stretch receptors) was assessed by the increase in expiratory time during end-inspiratory occlusion. The pulmonary chemoreflex (C-fiber endings) was assessed by the initial apnea and the secondary tachypnea after capsaicin intravenous injection. Results show the following. 1) Premature birth did not modify the maturation of the Hering-Breuer reflex. 2) Whereas a classic pulmonary chemoreflex was observed in the very first hours of life in preterm lambs, the tachypneic component of this reflex was weaker than in full-term lambs on day 1. 3) Premature birth led to a reversed postnatal maturation of this tachypneic response (tendency to increase with postnatal age). Our findings suggest that premature birth in lambs modifies postnatal maturation of the pulmonary chemoreflex.

neonatal respiratory control; slowly adapting bronchopulmonary stretch receptors; bronchopulmonary C-fiber endings; pulmonary chemoreflex; Hering-Breuer reflex

BY CONSTANTLY MONITORING THE mechanical status of the lungs throughout the breathing cycle, continuous vagal afferent information originating from various bronchopulmonary receptors allows the respiratory centers to alter and control lung volumes instantly (6). Results from previous studies have established that this vagal information is of crucial importance in the early postnatal period (19). Full-term lambs vagotomized either prenatally or at birth develop respiratory failure and die within the first 24 h of life (18). Although not so vitally important after 3–4 days of age, at least in precocious species such as lambs (25, 27), vagal afferent information is still of primary importance in the first weeks and months of life. Indeed, it is widely acknowledged that, conversely to the adult, Hering-Breuer inhibitory reflexes (HBIR) are functional within the tidal breathing range and exert a tight control on the breathing pattern in human infants (5, 29, 32). By doing so, vagal afferent input is especially important for dynamic maintenance of high end-expiratory lung volume, with the latter providing essential oxygen stores in the first months of life at a time when mechanical properties of the respiratory pump tend to decrease lung volume. In addition to HBIR activity, which is thought to originate from the slowly adapting bronchopulmonary stretch receptors, reflex activity originating from bronchopulmonary C-fiber endings may also be important for neonatal respiration. Bronchopulmonary C fibers have been previously shown to be functional as early as a few days of age in full-term newborn mammals, at least in the most precocious species such as pig (2) and sheep (9). As C-fiber endings are known to be stimulated by conditions encountered in the early neonatal period, such as pulmonary vascular congestion, increased interstitial lung water, and lactate ions, loss of their activity after vagal denervation may explain in part the onset of respiratory failure in vagotomized lambs at birth (18). Thus, whereas blockade of bronchopulmonary C fibers does not induce respiratory failure in postnatal lambs once they have reached a few days of age, it did prevent development of active expiratory laryngeal braking induced by increased interstitial lung water (9). However, to our knowledge, C-fiber function immediately at and/or after birth is totally unknown, especially in the preterm newborn mammal.

Our laboratory has recently developed an ovine model of the preterm newborn, which exhibits frequent central apneas and periodic breathing episodes, hence allowing us to describe laryngeal dynamics during neonatal apneas (30). This model also provides an excellent opportunity for studying postnatal development of vagal and chemical respiratory control in preterm vs. full-term newborns. The present study was aimed at assessing HBIR activity and cardiorespiratory responses, namely pulmonary chemoreflex (PCR), to serial intravenous (IV) injections of capsaicin from birth to 6 wk of life in nonsedated preterm vs. full-term lambs.

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METHODS

Animals

Ten preterm lambs (postconceptional age 132 ± 1 days, range 129–132 days) and seven full-term lambs (normal term 147 days) were studied. Mean birth weight was 2.9 ± 0.7 kg (range 1.9–4.3 kg) for preterm lambs and 4.4 ± 1.0 kg (range 3.1–5.8 kg) for full-term lambs. The protocol of the study was approved by our institution’s ethics committee for animal care and experimentation.

Preterm lambs were obtained as described in a previous study (30). Briefly, pregnancies were dated accurately by means of single mating after induction of estrus. Fetal lung maturation was accelerated by administration of betamethasone and thyrotropin-releasing hormone to the ewe within 48 h before delivery to prevent respiratory distress syndrome at birth. Vaginal delivery was induced by oxytocin IV infusion, 12 h after intravaginal placement of prostaglandin E2. Exogenous surfactant replacement (5 ml/kg; BLES, London, ON) was given to all lambs by direct intratracheal injection before the first breath. After standard initial neonatal care, the preterm newborn lamb was returned to its mother as soon as its general status, rectal temperature, glycemia, respiration, and transcutaneous oxygen saturation were normal and stable. In case of hypothermia (<38.5°C) and/or hypoglycemia (<2.3 mmol/l) because of insufficient spontaneous feedings, the lamb was warmed in an incubator and bottle-fed with mother’s milk. In cases of persistent retractions or grunting and/or tachypnea (<20 breaths/min) or arterial saturation <95%, despite supplemental nasal oxygen, a second dose of exogenous surfactant was given. One lamb needed endotracheal ventilatory support (Bourns-BP200, Life System, Riverside, CA) for 2 h within the first 24 h of life.

Full-term lambs were born in our local provider’s farm and arrived in our laboratory within 8 h of birth.

Study Design

An arterial catheter was inserted into the brachial artery under local anesthesia (2% lidocaine), 1 or 2 h before the first experiment. This catheter was left in place for the entire duration of the study and flushed daily with heparin solution. Antibiotics (25,000 U/kg im long-acting penicillin and 5 mg/kg im gentamicin) were repeated daily. A second catheter (InsysTe, 18GA, Infusion Therapy Systems) was introduced transcutaneously into the superior vena cava through the superficial jugular vein before each experiment. The lamb was then comfortably positioned in a sling with loose restraints. A plaster-molded face mask with a pressure port was glued on its snout with latex (26). The face mask was attached to a heated pneumotachograph (model 21070B plus, Hewlett-Packard, Waltham, MA, for lambs >3 kg, or model 8311, Hans Rudolph, Kansas City, MO, for lambs <3 kg). Instantaneous airflow, tidal volume (model 8815A, respiratory integrator, Hewlett-Packard), and mask pressure (MP 45-30-871, Validyne, Northridge, CA) were continuously monitored. Electrocardiogram (subcutaneous needle electrodes) and arterial blood pressure (pressure transducer Trantec model 60-800, American Edwards Laboratories, Santa Ana, CA; and pressure monitor model 7S42A, Hewlett Packard) were also monitored continuously. All variables were recorded on an Apple microcomputer with the use of data-acquisition software (AcqKnowledge version 3.2, MP100A, Biopac Systems, Santa Barbara, CA) and stored on compact disks for further analysis.

The following experiments were repeated at 24 h of age and then on days 4, 7, 14, 21, 28, and 42. All experiments were performed in nonsedated, conscious lambs during quiet room-air breathing and began with a 3-min baseline recording and arterial blood-gas measurement. Ambient temperature was kept between 20 and 22°C.

Ventilatory responses to stimulation of slowly adapting pulmonary stretch receptors (Hering-Breuer reflex). After the 3-min baseline recording, the Hering-Breuer reflex was induced by occluding the pneumotachograph at the end of a tidal inspiration. Two measurements were performed at a 1-min interval. The ensuing experiments (capsaicin injections) began 5 min after completion of the Hering-Breuer experiments.

Ventilatory and hemodynamic responses to stimulation of bronchopulmonary C-fiber endings (PCR). After the 3-min baseline recording, lambs were first given a 1-ml bolus IV injection of saline, followed by a 1-ml bolus IV injection of vehicle (Tween 80 + 10% ethanol), an equivalent dose to that used for 50 μg/kg capsaicin (Sigma Chemical, St Louis, MO). This was followed by capsaicin IV injections on each experimental day, according to the following protocol. Two doses of 5 μg/kg were first injected. If the ventilatory response was judged nonsignificant, two doses of 10 μg/kg were then injected, and so forth with two doses of 25 μg/kg and then two doses of 50 μg/kg. The experiment was halted at a given dose, when a 10-s apnea and/or a threefold increase in respiratory rate (RR) (= significant ventilatory response) was observed for at least 2 min (8). A minimum interval of 5 min was respected between all injections (volume = 1 ml), or until a return to baseline value.

In addition, the presence of PCR was assessed in three additional preterm lambs during the first 4 h after birth. Ventilation was assessed in these lambs by using respiratory inductance plethysmography; no mask or arterial catheter was used. One to two IV injections of 10 μg/kg capsaicin were administered in the three lambs.

Data Analysis

HBIR. The strength of the HBIR activity was quantified as follows. The expiratory time (Te) during the end-inspiratory occlusion (Teocc) and the control Te averaged from the three breaths preceding occlusion were first measured. The percentage of relative increase in Teocc (6Te/Teocc) was then calculated as [(Teocc − Te) × 100/Teocc] (32). Maturation of the strength of the HBIR activity was assessed by reporting the calculated values as a function of postnatal age for both preterm and full-term lambs.

PCR. All lambs received two injections of 5 μg/kg capsaicin. Only the lambs that had no significant response (see Study Design above) received higher doses. Analyses were performed at the 5 μg/kg dose and measured both components of the PCR, namely initial apnea with decrease in heart rate and arterial pressure and secondary tachypnea. For the initial portion of the reflex, the inhibitory ratio (IRcaps) was calculated as the ratio between apnea duration and baseline Te (averaged from the three preceding respiratory cycles). Moreover, analysis of the cardiovascular response included measurement of both minimal heart rate (HRmin) and mean arterial pressure (MAPmin) values during apnea, as well as baseline (control) heart rate (HRBL) and mean arterial pressure values (MAPBL). Baseline values were averaged over a 10-s period within the 20-s preceding capsaicin injection. The percentage of relative decrease in heart rate (%ΔHR) and in mean arterial pressure (%ΔMAP)
was calculated, respectively, as \( \frac{[\text{HR}_{\text{BL}}] - [\text{HR}_{\text{min}}]}{[\text{HR}_{\text{BL}}]} \times 100\% \) and \( \frac{[\text{MAP}_{\text{BL}}] - [\text{MAP}_{\text{min}}]}{[\text{MAP}_{\text{BL}}]} \times 100\% \).

For the second component of the PCR elicited by 5 μg/kg capsaicin injection, analysis of the increase in RR was performed by measuring baseline (RRBL) averaged over three cycles just before the injection and RR at 30, 60, and 120 s after injection. For example, the percentage of relative increase in RR at 60 s (RR60; %ΔRR60) was calculated as \( \frac{[\text{RR}_{60}] - [\text{RR}_{60}^0]}{[\text{RR}_{60}^0]} \times 100\% \).

Finally, the mean dose of capsaicin required for eliciting a 10-s apnea and/or a threefold increase in RR was considered as a significant response to capsaicin (8) and calculated at each age in both groups. For all of the above analyses, maturation of PCR was assessed by reporting the average calculated values as a function of postnatal age and postconceptional age (gestational age + postnatal age), for both preterm and full-term lambs.

**Statistical analysis.** Measurements were averaged for each lamb and then for the group as a whole for each experimental day. Group means were reported as means ± SD. Two-factor ANOVA for repeated measures (with mean comparison contrasts when applicable) was used to determine whether the response variables matured with age in each group and whether differences were present between group mean values at each age. In addition, covariance analyses adjusting for postnatal age were performed to compare both groups with respect to the response variables and to test whether maturation was different between both groups (SuperANOVA 1989, Abacus Concepts, Berkeley, CA). A global type I error of 5% was taken, and the Bonferroni correction was applied for multiple comparisons. We assumed that all of the response variables were normally distributed.

**RESULTS**

Out of a total of 10 preterm lambs, only six on day 1 and seven on day 4 were studied, because of supplemental oxygen needed to maintain normal blood oxygenation. Moreover, PCR could be studied in only six preterm and five full-term lambs on day 42, because of the development of continuous panting after a few minutes following completion of the Hering-Breuer experiments in the remaining lambs. Respiratory and cardiovascular parameter values measured in baseline conditions at all postnatal ages are reported in Table 1 for both full-term and preterm lambs. Similarly, arterial blood gases are reported in Table 2.

**HBIR**

Occlusion of the pneumotachograph at end inspiration led to a prolongation of T\( \text{E} \) in all studied lambs, regardless of age (Fig. 1). No statistically significant postnatal maturation of %ΔT\( \text{E} \) was found in either preterm (from 113 ± 94% on day 1 to 82 ± 66% on day 42; \( P \) between 0.0024 and 0.8933) or full-term lambs (from 97 ± 27% on day 1 to 82 ± 44% on day 42; \( P \) between 0.5663 and 0.9962; ANOVA for repeated measures). In addition, the postnatal evolution of %ΔT\( \text{E} \) in full-term and preterm lambs was not significantly different as measured by covariance analysis (\( \beta = -1.53, P = 0.0900 \)). Finally, no significant differences were found for %ΔT\( \text{E} \) between full-term and preterm lambs at any age (\( P \) between 0.0721 and 0.9838; ANOVA for repeated measures + contrast). Overall, premature birth was not found to modify postnatal maturation of the HBIR inspiratory reflex in lambs.

**PCR**

Injection of capsaicin led to a classic PCR, consisting of an apnea with bradycardia and systemic hypoten-

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**Table 1. Respiratory and cardiovascular parameter values in baseline conditions**

<table>
<thead>
<tr>
<th>Age</th>
<th>Respiratory Rate, breaths/min</th>
<th>Tidal Volume, ml/kg</th>
<th>Minute Ventilation, ml·kg⁻¹·min⁻¹</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Full term</td>
<td>Preterm</td>
<td>Full term</td>
<td>Preterm</td>
</tr>
<tr>
<td>D1</td>
<td>94 ± 8*</td>
<td>54 ± 16</td>
<td>9.6 ± 0.7</td>
<td>11.8 ± 1.5</td>
<td>901.0 ± 93.8*</td>
</tr>
<tr>
<td>D4</td>
<td>81 ± 18</td>
<td>53 ± 20</td>
<td>10.9 ± 2.0</td>
<td>9.3 ± 2.6</td>
<td>860.3 ± 133.3*</td>
</tr>
<tr>
<td>D7</td>
<td>95 ± 20*</td>
<td>50 ± 18</td>
<td>8.9 ± 1.0</td>
<td>10.6 ± 2.2</td>
<td>834.1 ± 134.1*</td>
</tr>
<tr>
<td>D14</td>
<td>70 ± 17</td>
<td>46 ± 17</td>
<td>9.3 ± 1.8</td>
<td>10.1 ± 2.1</td>
<td>620.7 ± 105.9</td>
</tr>
<tr>
<td>D21</td>
<td>68 ± 44*</td>
<td>35 ± 7</td>
<td>8.0 ± 2.3</td>
<td>9.8 ± 1.6</td>
<td>505.7 ± 265.1</td>
</tr>
<tr>
<td>D26</td>
<td>61 ± 38</td>
<td>44 ± 9</td>
<td>9.1 ± 4.1*</td>
<td>7.2 ± 1.6</td>
<td>485.5 ± 415.2*</td>
</tr>
<tr>
<td>D42</td>
<td>77 ± 20</td>
<td>39 ± 3</td>
<td>6.0 ± 1.1</td>
<td>7.5 ± 0.9</td>
<td>444.9 ± 88.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. D, day. *Significantly different from full term, \( P < 0.007 \).

**Table 2. Arterial blood gases in baseline conditions**

<table>
<thead>
<tr>
<th>Age</th>
<th>pH</th>
<th>P( \text{CO}_2 ) Torr</th>
<th>P( \text{O}_2 ) Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Full term</td>
<td>Preterm</td>
</tr>
<tr>
<td>D1</td>
<td>7.40 ± 0.02</td>
<td>7.38 ± 0.02</td>
<td>42.2 ± 7.0</td>
</tr>
<tr>
<td>D4</td>
<td>7.34 ± 0.04</td>
<td>7.37 ± 0.03</td>
<td>41.9 ± 7.0</td>
</tr>
<tr>
<td>D7</td>
<td>7.37 ± 0.06</td>
<td>7.40 ± 0.02</td>
<td>39.3 ± 3.2</td>
</tr>
<tr>
<td>D14</td>
<td>7.36 ± 0.03</td>
<td>7.39 ± 0.03</td>
<td>40.6 ± 3.8</td>
</tr>
<tr>
<td>D21</td>
<td>7.38 ± 0.04</td>
<td>7.37 ± 0.02</td>
<td>37.6 ± 4.4</td>
</tr>
<tr>
<td>D28</td>
<td>7.40 ± 0.02</td>
<td>7.37 ± 0.02</td>
<td>38.1 ± 4.9</td>
</tr>
<tr>
<td>D42</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.04</td>
<td>37.1 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. pH, arterial pH; P\( \text{CO}_2 \), arterial P\( \text{CO}_2 \); P\( \text{O}_2 \), arterial P\( \text{O}_2 \).
sion, followed by tachypnea, in all studied lambs (Fig. 2), including the three preterm lambs challenged within 4 h after birth.

Initial portion of the PCR. On day 1, all studied lambs exhibited a central apnea immediately after injection of 5 μg/kg capsaicin. No statistically significant postnatal maturation of IRcaps was found in preterm lambs (from 16.3 ± 14.4 on day 1 to 11.3 ± 10.6 on day 42, P between 0.0379 and 0.9155) (Fig. 3A). Conversely, IRcaps was found significantly different in full-term lambs between days 21 and 42 (from 11.4 ± 8.9 on day 1 to 7.2 ± 5.1 on day 21 and 25.9 ± 19.5 on day 42; P = 0.0019 between days 21 and 42; ANOVA for repeated measures). In addition, the postnatal evolution of IRcaps was significantly different in full-term and preterm lambs by covariance analysis (β = 0.4, P = 0.0100). Finally, no significant differences were found for IRcaps between full-term and preterm lambs at any age (P between 0.0145 and 0.9500; ANOVA for repeated measures + contrast). Overall, the postnatal increase in apneic response to capsaicin injection observed in full-term lambs (especially between postnatal days 21 and 42) was blunted in preterm lambs.

A decrease in HR and MAP was part of the initial response to injection of 5 μg/kg capsaicin in all studied lambs and at all ages (Fig. 3, B and C). Overall, the %ΔHR ranged from 33 to 71% for preterm lambs and from 43 to 70% for full-term lambs. Furthermore, the %ΔMAP ranged from 11 to 21% for preterm lambs and from 8 to 34% for full-term lambs. Both %ΔHR and %ΔMAP returned to ±7% baseline values within 10 s after apnea in all lambs and at all ages studied. Covariance analysis did not reveal any significant differences between full-term and preterm lambs in the postnatal maturation of both %ΔHR (β = −0.3, P = 0.3574) and %ΔMAP (β = 0.1, P = 0.7182). Whereas %ΔHR was statistically significantly greater in full-term than in preterm lambs on days 14 and 28 (P = 0.0019, ANOVA for repeated measures + contrast), this trend was highly variable within the study period (Fig. 3B). No significant postnatal maturation was found for %ΔMAP in either preterm or full-term lambs, and neither were any differences observed between preterm and full-term lambs at any age for %ΔMAP (ANOVA for repeated measures + contrast) (Fig. 3C). Overall, premature birth did not alter postnatal maturation of the cardiovascular responses to capsaicin injection.

Secondary tachypnea. After apnea, most lambs exhibited a central apnea immediately after injection of 5 μg/kg capsaicin. No statistically significant postnatal maturation of IRcaps was found in preterm lambs (from 16.3 ± 14.4 on day 1 to 11.3 ± 10.6 on day 42, P between 0.0379 and 0.9155) (Fig. 3A). Conversely, IRcaps was found significantly different in full-term lambs between days 21 and 42 (from 11.4 ± 8.9 on day 1 to 7.2 ± 5.1 on day 21 and 25.9 ± 19.5 on day 42; P = 0.0019 between days 21 and 42; ANOVA for repeated measures). In addition, the postnatal evolution of IRcaps was significantly different in full-term and preterm lambs by covariance analysis (β = 0.4, P = 0.0100). Finally, no significant differences were found for IRcaps between full-term and preterm lambs at any age (P between 0.0145 and 0.9500; ANOVA for repeated measures + contrast). Overall, the postnatal increase in apneic response to capsaicin injection observed in full-term lambs (especially between postnatal days 21 and 42) was blunted in preterm lambs.

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Secondary tachypnea. After apnea, most lambs exhibited a bout of agitation during a few seconds (<5 s) immediately preceding onset of tachypnea. The %ΔRR60 after injection of 5 μg/kg capsaicin was significantly higher in full-term than in preterm lambs on
Moreover, although the tendency for increasing \%ΔRR_{60} in preterm lambs (from 19 ± 17\% on day 1 to 212 ± 108\% on day 42) and decreasing \%ΔRR_{60} in full-term lambs (from 318 ± 224\% on day 1 to 167 ± 173\% on day 42) was not significant (ANOVA for repeated measures), there was a significant difference in postnatal maturation of \%ΔRR_{60} between preterm and full-term lambs by covariance analysis (β = 6.59, P = 0.0155). Similar trends were found for \%ΔRR at 30 and 120 s for both preterm and full-term lambs. Overall, whereas premature birth blunted the tachypneic response to capsaicin injection on day 1, it was responsible, however, for altering its postnatal maturation toward an increased response, conversely to the decreasing trend in full-term lambs.

Fig. 4. Secondary tachypnea induced by IV injection of 5 μg/kg capsaicin in preterm (○) vs. full-term (●) lambs, plotted as a function of postnatal (A) and postconceptional age (B). Values are means ± SD. *Significant difference preterm vs. full-term lambs, P < 0.007.
Dose of capsaicin necessary for eliciting a significant ventilatory response. Analysis of the mean dose of capsaicin required for eliciting a “significant” ventilatory response (a 10-s apnea and/or a threefold increase in RR) revealed that it was significantly higher in preterm lambs only on day 1 ($P = 0.0071$; ANOVA for repeated measures + contrast). The mean dose consistently tended to be higher in preterm lambs throughout the study period. No significant effect of postnatal maturation was found in either group (ANOVA for repeated measures). Finally, no significant difference in postnatal maturation was observed between preterm and full-term lambs by covariance analysis ($\beta = 0.08, P = 0.6588$) (Fig. 5 and Table 3). Overall, premature birth was responsible for increasing the dose of capsaicin necessary to elicit a significant PCR on day 1. There was no statistically significant differences thereafter.

Early testing in preterm lambs. Apnea duration in the three preterm lambs challenged with 10 $\mu$/kg capsaicin within 4 h of life was 6.9 ± 0.9 s (range 5.6–7.7 s). Moreover, whereas the increase in minute ventilation was not measured in these lambs (no face mask), a tachypneic response was observed in all three lambs, with a mean increase in $RR_{60}$ postinjection of 145 ± 83%.

DISCUSSION

Results of the present study provide new insight into the development of vagal cardiopulmonary reflexes originating from bronchopulmonary receptors in preterm vs. full-term lambs during the first 6 wk of life. First, it was found that premature birth did not alter HBIR activity at birth or its postnatal maturation in preterm vs. full-term lambs. Second, we describe for the first time that the classic PCR, induced by capsaicin IV injection, is already present in the very first hours of life in preterm lambs. Furthermore, whereas the tachypneic component of PCR is more important in full-term lambs at birth, postnatal maturation of this tachypneic response is reversed in preterm vs. full-term lambs.

Importance of Vagal Innervation for Respiratory Adaptation at Birth

Vagal innervation has been shown to be of vital importance at birth in full-term lambs. Indeed, bilateral vagal denervation right after birth leads to venti-
latory failure and death in 24 h. The underlying mechanisms of reduced pulmonary compliance and hypoxemia include progressive atelectasis, resulting from the absence of expiratory braking, low RR, prolonged T_{E}, and decrease in the frequency of sighs (18, 33). Demonstration of the vital importance of vagal innervation at birth establishes the relevance of the present study, which is aimed at a better understanding of the various components of vagal afferent activity in the neonatal period.

**HBIR**

Since the first description of the Hering-Breuer reflexes in 1868, measurement of the strength of these reflexes has become the accepted method for assessing the function of slowly adapting bronchopulmonary stretch receptors. Brief end-inspiratory occlusion has been invariably reported to delay the onset of the next inspiratory effort in newborns of various species, including preterm infants (21). Results of the present study showing no change in HBIR activity in full-term lambs during the first 6 wk of life are in agreement with previous findings in full-term infants (28, 32). Interestingly, whereas it has been suggested that HBIR activity is higher in quiet sleep than during wakefulness, values of HBIR activity in this study in awake full-term lambs are similar to values previously reported in infants during quiet sleep, either natural or induced by light sedation (32). Thus, despite increased neurological maturation in lambs and wakefulness, both of which normally decrease HBIR activity, HBIR activity was found to be similar in full-term lambs and infants.

Studies aimed at assessing the influence of premature delivery on HBIR activity in human infants have produced conflicting results (3, 11, 12, 17, 23, 32, 34). The following observations can be made from the present results. First, HBIR activity is similar at birth in both preterm and full-term lambs, which is in agreement with previous results in infants, with identical values observed in infants and lambs (33). Second, there is no change in HBIR activity with postnatal age in preterm lambs. On the other hand, previous results in preterm infants showed that HBIR activity increased from 1 to 5 wk after birth, before progressively decreasing afterward (32, 34). At present, there is no explanation for these discrepancies. Overall, our results suggest that premature birth does not alter postnatal maturation of HBIR activity. Moreover, the high HBIR activity found in the first 6 wk of life in both preterm and full-term lambs confirms that monitoring of lung volumes throughout tidal ventilation by slowly adapting bronchopulmonary stretch receptors is also an important component of neonatal respiratory control in sheep, allowing the regulation of breathing patterns and maintenance of an optimal end-expiratory lung volume (15).

**PCR**

Brodie (4) was the first to describe a complex cardiorespiratory response, which included bradycardia, hypotension, and apnea followed by rapid, shallow breathing and bronchoconstriction, after IV injection of horse serum in cats. Since then, various chemicals, including phenyl diguanide, capsaicin (8), and lactic acid (20, 22), have been shown to evoke a PCR in numerous species, including conscious adult sheep (7). The initial component of the PCR, consisting of an apnea with bradycardia and hypotension, is most likely due to the abrupt simultaneous stimulation of the “total” population of pulmonary C-fiber endings (13, 24). The second component of the PCR, i.e., tachypnea, has also been shown to be related to stimulation of pulmonary C-fiber endings (13). A dose-dependent effect has been described in spontaneously breathing cats, with smaller doses producing only rapid, shallow breathing and larger doses being needed to elicit apnea (1). This was not confirmed, however, in the present study, in either preterm or full-term lambs or in a previous study in dogs (13).

**PCR in the newborn mammal.** Previous studies of either the two components of PCR (8, 31) or secondary bronchoconstriction alone (2, 22) have shown that the PCR can be consistently induced by chemical injection of capsaicin, phenyl diguanide, or lactic acid in newborn mammals. Available studies suggest that vagal reflexes originating from bronchopulmonary C-fiber endings in the newborn are similar to those observed in the adult mammal, albeit with a higher threshold in the less precocious species (10, 21). No data are available, however, during the first day of life.

**Initial component of the PCR.** Our findings suggest that normal maturation of the initial component of the PCR in full-term lambs is depicted by an increase in the duration of apnea, reflecting an increase in the maximal respiratory response of pulmonary C fibers. A decrease in the proportion of nonmyelinated fibers in the vagus from fetal to full-term lambs (16) suggests that the increase in apneic response, which we observed with age, is merely due to enhanced afferent neuronal transmission and/or central integration. This is in keeping with the higher threshold for elicitation of PCRs reported previously (10, 21). The reason why bradycardia and hypotensive responses remained unchanged with maturation in full-term lambs is unknown. In preterm lambs, no change with maturation was observed for apnea duration, suggesting an abnormal postnatal maturation of C-fiber function. Results for bradycardia or systemic hypotension were identical (no change with postnatal age) in preterm and full-term lambs.

**Secondary tachypnea.** Importance of the tachypneic response is related to the hypothesis that it is more representative of physiological C-fiber function than the initial cardiorespiratory depression (13). Overall, results from the present study suggest that the tachypneic response is higher at birth in full-term than in preterm lambs. Moreover, postnatal maturation of the tachypneic response in full-term lambs has a tendency to decrease with age, which is reversed by premature delivery. Although there is presently no explanation for these findings, the abnormal postnatal maturation...
of pulmonary C-fiber function in preterm lamb is remarkable. Whether our findings may be part of the puzzle explaining the higher incidence of sudden infant death syndrome in former preterm infants remains unknown at this stage.

The need for a higher dose of capsaicin to elicit a similar response in preterm lambs compared with full-term lambs may appear at odds with the reported higher proportion of unmyelinated fibers in the immature vagus nerve (16, 21). At least two factors could explain this apparent discrepancy. First, unmyelinated fibers in immature nerves comprise not only C fibers, but also future myelinated fibers, albeit in proportions unknown. Second, it is likely that central integration of C-fiber trafficking of afferent messages operates at a higher threshold (21).

Elicitation of the PCR in the first hours of life. The unique findings of a PCR elicited by capsaicin IV injection in preterm lambs within the first 4 h of life are especially relevant to the clinical situation of the preterm infant. Indeed, the various conditions known to stimulate bronchopulmonary C fibers, including metabolic acidosis accompanying normal parturition and inflammatory lung disease (22), pulmonary vascular congestion (21), and increased lung water content (21), are frequently present in the neonatal period. Moreover, our laboratory has previously shown that bronchopulmonary C-fiber endings are crucial for controlling laryngeal bracing of expiratory airflow (expiratory grunting) (9), which is an important defense mechanism of the newborn, enabling it to preserve oxygenation in the very first hours of life (14). Taken together, the present findings, as well as previous data, suggest that reflexes arising from the bronchopulmonary C-fiber endings are important from birth in full-term and preterm infants, both for normal respiratory adaptation to extraterine life and for maintaining oxygenation in case of respiratory distress syndrome.

In conclusion, the present findings suggest that premature birth does not alter postnatal maturation of the reflex activity arising from bronchopulmonary slowly adapting stretch receptors during the first 6 wk of life in lambs. Moreover, our results bring unique information on the reflex activity arising from bronchopulmonary C-fiber endings and its postnatal maturation. This includes the reverse maturation of the tachypneic response in preterm lambs (= tendency to increase with postnatal age), and the demonstration that reflex activity from C-fiber endings is present in the very first hours of life in preterm lambs. The importance of these findings is in keeping with the vital necessity of vagal innervation at birth.

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