Periodic breathing induced on demand in awake newborn lamb

EMMANUEL CANET, JEAN-PAUL PRAUD, AND MICHEL A. BUREAU
Unité de Recherche Pulmonaire, Département de Pédiatrie, Université de Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4

Canet, Emmanuel, Jean-Paul Praud, and Michel A. Bureau. Periodic breathing induced on demand in awake newborn lamb. J. Appl. Physiol. 82(2): 607–612, 1997.—Spontaneous periodic breathing, although a common feature in fullterm and preterm human infants, is scarce in other newborn mammals. The aim of this study was to induce periodic breathing in lambs. Four 10-day-old and two <48-h-old awake lambs were instrumented with jugular catheters connected to an extracorporeal membrane lung aimed at controlling arterial PCO2 (PaCO2). Arterial PO2 (PaO2) was set and maintained at the desired level by changing inspired O2 fraction and providing O2 through a small catheter into the “apneic” lung. At a critical PaCO2/PaO2 combination, the four 10-day-old lambs exhibited periodic breathing that could be initiated, terminated, and reinitiated on demand. In the 2-day-old lambs with low chemoreceptor gain, periodic breathing was hardly seen, regardless of the trials done to find the critical PO2/PCO2 combination. We conclude that periodic breathing can be induced in lambs and depends on critical PaO2/PaCO2 combinations and maturity of the chemoreceptors.

METHODS

Experimental subjects. Six Rambouillet-Suffolk lambs, born by spontaneous vaginal delivery at sea level and housed in our animal quarters, were used in the study. Two lambs were between 12 and 48 h of age and four were between 10 and 12 days of age. The study protocol was approved by the ethics committee for animal research at our institution.

Design of study. The experimental design allowed us to set the PaCO2 and PaO2 at levels expected to induce sustained periodic breathing by using two gas exchangers connected in series (7, 15). The first gas exchanger, an extracorporeal membrane lung connected to the lamb by a venovenous shunt, was used for adjustment of PaCO2; the second gas-exchanger was the lamb's lung, used for adjustment of PaO2. At constant PaO2, PaCO2 could be altered by changing gas flow or CO2 fraction through the extracorporeal membrane. Conversely, at constant PaCO2, PaO2 could be altered by changing the inspired O2 fraction (FiO2) of the lamb's lung or by supplying humidified and heated O2 in small quantities (25–100 ml/min) by using a polyvinyl 5-Fr catheter introduced in the endotracheal tube beyond 1 cm of its tip.

After surgical preparation (see below), the lamb was placed comfortably in a prone position on an animal cart under...
gentle restraint that did not interfere with chest or abdominal movement. The first baseline ventilatory measurements and arterial blood-gas samplings were performed during a 2-min period during normal room-air breathing. The animal was then connected to the extracorporeal circuit (progressively set to obtain a constant blood flow) while no gas flowed through the membrane (no gas exchange). A minimum of 30 min was allowed for the lamb to become accustomed to the venovenous shunt and the blood flowing through the membrane. The gas flow through the membrane was then opened, and the critical $P_{aO_2}/P_{aCO_2}$ values for periodic breathing were searched as follows.

First, based on our previous results (6), we tested whether a decrease in $P_{aCO_2}$ (approximately to the 25–35 Torr range) and $P_{aO_2}$ (approximately to the 30–40 Torr range) would lead to the development of periodic breathing. Gas flow to the membrane was set to a value expected to lead to the aimed $P_{aCO_2}$ range, and $F_{I_2}$ delivered to the lamb's lung was decreased to 10%. If necessary, these parameters were then slightly modified in a stepwise fashion (most often empirically) until periodic breathing developed. At least 10 min were allowed for stabilization between each modification. Critical $P_{aO_2}/P_{aCO_2}$ values were measured in arterial blood systematically drawn at the onset of periodic breathing.

Second, because one of our objectives was to evaluate the gap between $P_{aCO_2}$ at the onset of periodic breathing and $P_{aCO_2}$ at the point of apnea, we further determined the apneic threshold in each lamb by decreasing $P_{aCO_2}$ to the point of apnea as previously described (7). $P_{aCO_2}$ was kept constant during this procedure by supplying small quantities of O₂ via the endotracheal tube catheter on the basis of blood gas analysis obtained from frequent arterial blood withdrawal. By anticipating occurrence of apnea, we were able to precisely determine the $P_{aCO_2}/P_{aCO_2}$ combination that resulted in apnea. After a few minutes of apnea, gas flow through the membrane was discontinued, thereby allowing for spontaneous ventilation of breathing.

Finally, we tested the general hypothesis that periodic breathing is the result of disturbing an unstable respiratory chemical system. Because significant association between periodic breathing and sighs has been reported in human infants, we tried to induce periodic breathing by mimicking a sigh. After adjusting $P_{aCO_2}$ and $P_{aO_2}$ near critical $P_{aO_2}/P_{aCO_2}$ values for periodic breathing, one sigh breath (the volume of which was at least twice the tidal volume (VT)) was induced by bagging the lamb with a hand bag connected to the endotracheal tube.

Surgical preparation. Each lamb was prepared surgically, as reported in detail elsewhere (7). Briefly, 3 h before the experiment, after applying a 2% lidocaine spray to the oropharynx and larynx, a percutaneous intubation was performed. An appropriately sized (4.5–6.0 mm) endotracheal tube was inserted, with cuff pressure maintained at 25 cmH₂O; then, under sterile conditions and local anesthesia (2% lidocaine), the catheters were put in place. An arterial catheter was inserted into the innominate artery for arterial blood-gas sampling and for monitoring systemic blood pressure and heart rate. After administering a loading dose of heparin (300 U/kg), the animal's coagulation status was monitored throughout the experiment via the Hemochron apparatus (International Technidyne) so that an activating clotting time could be maintained in the range of 180–300 s. Venous cannulations were performed for connection to the extracorporeal circuit through the jugular veins by using thin-walled spring-wire-reinforced polyurethane catheters (Bypass Systems). One cannula was inserted through the left jugular vein (12 Fr) and advanced to the superior vena cava for use as the venous return line. A second cannula (12 Fr) was inserted through the right jugular vein and advanced centrally to 1 cm above the diaphragm to allow drainage of both the inferior vena cava and the atrium through lateral side holes. Cannulas (12 Fr) were inserted into each jugular vein in a cephalad direction to allow for drainage of the head on each side. Positioning of the catheters was controlled by X ray of the neck and chest. Due to the easy accessibility of the jugular veins in the lamb, all catheters could be introduced under local anesthesia (2% lidocaine) without any undue discomfort to the animal.

Extracorporeal circuit. As described previously (7), a venous-to-venous extracorporeal bypass circuit was used. The venous blood was drawn by gravity from the atrium, inferior vena cava, and both head-drainage jugular cannulas into a 50-ml reservoir bag that, in turn, activated a servo-controlled centrifugal pump (Biomedicus). From the centrifugal pump, the blood passed through one membrane lung (4.5 m² surface area, Bentley) and then returned to the lamb through the left venous catheter to the superior vena cava. A heat exchanger affixed to the membrane lung was adjusted to enable the lamb's rectal temperature to be maintained at its baseline value (39.5°C) during the entire experimental procedure. Gas flow to the membrane lung was supplied from a cylinder and regulated with precision rotameters. The gas was heated to 37°C and humidified before flowing through the membrane lung.

On the morning of the experiments, the extracorporeal perfusion circuit was primed with Ringer-lactate solution and subsequently displaced with homologous sheep blood (800 ml) containing 5 units of heparin/ml. The blood, recirculated through the circuit for 1 h before any connection was made to the lamb, was adjusted to approximate normal venous pH as well as venous $P_{aO_2}$ and $P_{aCO_2}$. Aseptic procedures were followed throughout the experiment. Once the circulated blood was conditioned, the lamb was connected to the circuit and the blood flow through the pump was slowly increased to $\sim$150 ml·min⁻¹·kg⁻¹ and maintained at this level throughout the entire experiment.

Ventilatory measurements. The endotracheal tube was connected to a size 0 pneumotachograph (Hewlett-Packard 21070B, Pomona, CA). The resulting flow signal, integrated with respect to time to give volume (model 8815A Hewlett-Packard respiratory integrator), was recorded on a strip-chart recorder. End-tidal $P_{aO_2}$ and $P_{aCO_2}$ were sampled from the endotracheal tube and analyzed by a mass spectrometer (model MGA-1100, Perkin-Elmer). Simultaneously, the pneumotachograph flow signal and the mass spectrometer tidal $P_{aO_2}$ and $P_{aCO_2}$ signals were sent to a PDP-11/23 computer (Digital Equipment) for a breath-by-breath computer analysis. The flow wave, recorded by an analog-to-digital converter, was analyzed at a sampling rate of 40 Hz. The collected signals were stored on disk for further analysis. The respiratory variables derived by the computer for each breath were minute ventilation; VT; respiratory rate (f); inspiratory time (TI), expiratory time, and total time of each cycle; mean inspiratory flow (VT/TI), duty cycle (IT/total time), end-tidal $P_{aO_2}$, and end-tidal $P_{aCO_2}$. For blood-gas analysis, duplicate 1-ml samples were taken anaerobically into 3-ml syringes and analyzed immediately for $P_{aO_2}$, $P_{aCO_2}$, and pH determinations (Micro 13, Instrument Laboratory).

Definition of periodic breathing. For this study, periodic breathing was defined as cyclic fluctuations of respiratory efforts interrupted by periods of apnea ($\geq$3 s) or hypopnea in a crescendo-decrescendo pattern.
RESULTS

The fact that the lambs were connected to an extracorporeal circuit set at a constant blood flow (while the membrane was closed to gas flow) had no effect on baseline minute ventilation or on arterial blood gases compared with baseline values (Table 1). The absence of cyclic fluctuation in ventilation confirmed that this procedure, by itself, did not affect the stability of the ventilatory controller.

Critical PaO₂/PaCO₂ for periodic breathing in 10-day-old lamb. By decreasing PaCO₂ and PaO₂ to values derived from previous results, we were successful in finding critical PaO₂/PaCO₂ combinations that would induce periodic breathing in the four 10-day-old lambs, i.e., periodic breathing with central apneas in three lambs and periodic breathing with hypopneas in the remaining lamb. Critical values of arterial blood gases at onset of periodic breathing are given in Table 2. It was found that once the periodic breathing cycle was initiated, periodic breathing could be maintained for any time period desired (Figs. 1 and 2). Periodic breathing could also be turned off by changing PaO₂/PaCO₂, and reinitiated by returning to the critical blood-gas tensions. As expected, periodic oscillations in breathing were brought about by changes in both VT and f. This phenomenon is illustrated in Fig. 2, in which breath-by-breath changes in minute ventilation, VT, f, and corresponding changes in end-tidal PaCO₂ and PaO₂ are given.

Relationship between periodic breathing and point of apnea. As shown in Table 2, PaCO₂ at onset of periodic breathing and PaCO₂ at the point of apnea were separated by only 1–3 Torr (Table 2). Consequently, periodic breathing could also be initiated in hypoxia while bringing the animal to apnea by slowly decreasing the PaCO₂ level (Fig. 3A).

In addition, after a few minutes of apnea with hypoxia, when gas flow in the membrane was closed, the apneic lamb accumulated CO₂ and resumed ventilation with cycles of periodic breathing (Fig. 3B).

Sighs and periodic breathing. Once gas tensions were set in the vicinity of the critical PaO₂/PaCO₂ combination for periodic breathing, the lamb was given a large breath, mimicking a sigh. This resulted in apnea and periodic breathing cycles (Fig. 4A). Additionally, in the same PaO₂/PaCO₂ conditions, a spontaneous sigh was observed followed by an episode of periodic breathing (Fig. 4B).

Termination of periodic breathing. Methods to terminate periodic breathing were tested by manipulating FIO₂ to the lamb’s lung or CO₂ fraction of the gas flowing through the membrane. In all instances, addition of 3% CO₂ in air through the extracorporeal membrane invariably terminated periodic breathing (Fig. 5A). In much the same way, periodic breathing could be stopped within a few bursts of pure O₂, offsetting the chemoreceptor function and allowing the CO₂ drive to control breathing (Fig. 5B).

Effect of age. In the four 10-day-old lambs, we were able to induce sustained periodic breathing on demand. Conversely, in the two 2-day-old lambs, the same attempts to produce periodic breathing were less suc-

Table 1. Baseline ventilatory and blood gas data in instrumented animals with and without extracorporeal circulation

<table>
<thead>
<tr>
<th></th>
<th>10-Day-Old Lambs</th>
<th>2-Day-Old Lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vi, ml·min⁻¹·kg⁻¹</td>
<td>pH</td>
</tr>
<tr>
<td>Lamb 1</td>
<td>Off</td>
<td>486</td>
</tr>
<tr>
<td></td>
<td>On</td>
<td>443</td>
</tr>
<tr>
<td>Lamb 2</td>
<td>Off</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td>On</td>
<td>459</td>
</tr>
<tr>
<td>Lamb 3</td>
<td>Off</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td>On</td>
<td>411</td>
</tr>
<tr>
<td>Lamb 4</td>
<td>Off</td>
<td>336</td>
</tr>
<tr>
<td></td>
<td>On</td>
<td>391</td>
</tr>
</tbody>
</table>

Vi, minute ventilation; PaCO₂, arterial Pco₂; PaO₂, arterial PO₂; Off, without extracorporeal circulation; On, with extracorporeal circulation.

Table 2. Arterial blood gas values during baseline recordings, at onset of PB, and at onset of apnea

<table>
<thead>
<tr>
<th></th>
<th>Baseline PaO₂, Torr</th>
<th>PB PaO₂/PaCO₂, Torr/Torr</th>
<th>Apnea PaO₂, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb 1</td>
<td>37</td>
<td>29.2/40.2</td>
<td>27.8</td>
</tr>
<tr>
<td>Lamb 2</td>
<td>40.9</td>
<td>23.6/30.8</td>
<td>22.8</td>
</tr>
<tr>
<td>Lamb 3</td>
<td>38.1</td>
<td>22.6/28</td>
<td>19.5</td>
</tr>
<tr>
<td>Lamb 4</td>
<td>41.5</td>
<td>38/29.5</td>
<td>34.3</td>
</tr>
</tbody>
</table>

Baseline values were taken in normoxia. PB, periodic breathing.
cessful. In one lamb, periodic breathing was never observed, whereas in the other it was not sustained.

**DISCUSSION**

This study shows that respiratory controllers in awake lambs can produce periodic breathing. Periodic breathing requires the same two conditions as in other mammals, one being the critical \( P_{A\text{O}_2} / P_{A\text{CO}_2} \) combination, the second being a sufficient state of maturity of the chemoreceptors to allow for high-chemoreceptor gain when the critical blood-gas level is reached.

The data obtained in the present study are interesting with regard to one point. They establish without a doubt that the periodic breathing of awake lambs is a true chemoreceptor event. Given the critical combination of \( P_{A\text{O}_2} / P_{A\text{CO}_2} \), in the lambs, we were able to produce periodic breathing on demand, maintain periodic breathing, stop periodic breathing, even reinitiate periodic breathing. In addition, at critical \( P_{A\text{O}_2} / P_{A\text{CO}_2} \) values, by maneuvers known to change blood-gas tensions and, consequently, chemoreceptor output (such as a sigh, a sharp decrease or increase in \( F_{\text{I}O_2} \), or a burst of increased inspired \( \text{CO}_2 \) fraction), predictable results with respect to enhancement or stoppage of periodic breathing could be elicited. These data clearly establish that periodic breathing can be induced in awake newborn lambs and that it critically depends on the chemical “drive to breathe.” We have not, as yet, tested the potentially modulatory effect of sleep state on periodic breathing.

The difficulty of producing periodic breathing in the less-than-2-day-old lambs deserves comments. At this age, prior studies from our laboratory (5) and others (3, 21) have shown that the chemoreceptors are still set to

**Fig. 2.** Graphical representation of time-related changes in minute ventilation (\( V_I \)), \( V_T \), respiratory frequency (\( f \)), end-tidal \( P_{\text{O}_2} \) (PETO\(_2\)) and end-tidal \( P_{\text{CO}_2} \) (PETCO\(_2\)) during sustained periodic breathing in 12-day-old lamb from Fig. 1. Each point represents 1 breath.

**Fig. 3.** Relationship between point of apnea and periodic breathing (hypoxic conditions). A: during extracorporeal \( \text{CO}_2 \) removal (ECCO\(_2\)R), progressive decrease in \( P_{\text{ACO}_2} \) toward apnea point induces periodic breathing, which soon progresses to sustained apnea. B: breathing resumption after cessation of ECCO\(_2\)R leads to periodic breathing, rapidly replaced by stable breathing pattern when \( P_{\text{ACO}_2} \) increases further from apnea point.
respond to very low levels of $\text{PaO}_2$ (3) and high levels of $\text{PaCO}_2$ (18). In other words, immediately after birth, as witnessed by the 2-day-old lambs, the chemoreceptors are not capable of eliciting a sufficiently brisk chemoreflex response to blood-gas changes to produce sustained periodic breathing. After birth, the chemoreceptors have to adapt to new blood-gas values; they must shift to new response thresholds to these blood gases. In the process, they acquire sufficient oxystatic and capnostatic chemoreceptor gains to produce sustained periodic breathing when critical blood gas tensions are reached. In lambs, the maturation process of the chemoreceptors is progressive over the first 2 wk of life (3, 5). It is therefore not surprising that sustained periodic breathing could not be induced in our younger lambs inasmuch as they had not developed their capacity of high chemoreceptor gain. One could argue that the window of critical $\text{PaO}_2/\text{PaCO}_2$ levels needed to produce this periodic breathing does exist at this age, but that we were unable to find it in our numerous attempts to identify the critical blood-gas combinations. In our view, however, this supposition is unlikely. We believe that newborns in the first days of life, as well as fetuses in prenatal life, simply do not have a sufficiently high chemoreflex gain to produce sustained periodic breathing. This is in agreement with our previous study of posthyperventilation breathing oscillations in lambs (6), showing that 2-day-old lambs, because of lower chemoreceptor responsiveness, did not experience as much periodic breathing as did 10-day-old lambs.

The relationship between the critical $\text{PaCO}_2$ that produces periodic breathing and the critical $\text{PaO}_2$ that produces apnea is interesting. Those two critical points are separated by only 1–3 Torr. This means that when $\text{PaCO}_2$ is at 2–3 Torr from the apnea point, large variations in breathing, as a sigh, could reach the point of apnea and induce periodic breathing, provided that $\text{PaO}_2$ is also low enough to increase the chemoreceptor gain.

Despite differences between species, extrapolation of our findings regarding periodic breathing in lambs to periodic breathing in human neonates is warranted. The difficulty of producing periodic breathing in lambs during the first 2 days of life is in agreement with the current literature dealing with human infants. We have observed that at that age, periodic breathing simply does not exist in human infants (4); it occurs later, at a time when chemoreceptors have been reset for activation at a higher level of responsiveness. Similarly, Barrington and Finer (1) demonstrated that the propensity of human infants to develop periodic breathing is both age related and associated with chemoreceptor maturation. Additionally, in our lambs, when $\text{PaCO}_2/\text{PaO}_2$ was set at the appropriate critical level, a simple sigh or passive ventilation produced periodic breathing. Along the same lines, Bureau et al. (4) have reported that spontaneous periodic breathing in human neonates is often preceded by a brief period of hyperpnea believed to lower $\text{PaCO}_2$ toward the critical level for periodic breathing; a simple sigh could then initiate periodic breathing in infants (11). Moreover, in both species, inhalation of $\text{O}_2$ ends periodic breathing.

Hence, periodic breathing of both newborn lambs and human neonates seems to be a chemoreceptor-driven event, although quantitative differences between these species are still remarkable. For example, in human infants, a few large breaths or a sigh are enough to bring infants to the critical $\text{PaO}_2/\text{PaCO}_2$ window needed to induce periodic breathing. This phenomenon implies that the breathing of human infants is naturally set at
a blood-gas level close to the level needed for periodic breathing. In contrast, in awake lambs, much like adults, the critical $P_{O_2}/P_{CO_2}$ window required to induce periodic breathing is much lower than the baseline $P_{O_2}/P_{CO_2}$, and drastic experimental conditions are needed to reproduce periodic breathing. It is therefore not surprising that lambs do not have spontaneous periodic breathing, whereas most healthy human infants do, even though their mechanisms leading to periodic breathing are alike. The fact that our studies were conducted in awake animals might also have contributed to this discrepancy. The effect of sleep on ventilatory responses to chemical stimuli is well established, and recurring changes in sleep state could be considered as a contributing factor that may promote ventilatory instability.

In conclusion, this study shows that it is possible to produce periodic breathing on demand in awake lambs when appropriate settings of blood gases are reached to drive the chemoreceptors. It also strongly suggests that, in newborn mammals, periodic breathing is a chemoreceptor-mediated event.

The authors acknowledge the expert secretarial assistance of Marguerite Cloutier and the technical assistance of D. Edgell. This research was supported by Medical Research Council of Canada Grant MT7137. J.-P. Praud is a scholar of the Fonds de la Recherche en Santé du Québec, Canada Grant MT7137. J.-P. Praud is a scholar of the Fonds de la Recherche en Santé du Québec. The authors acknowledge the expert secretarial assistance of Marguerite Cloutier and the technical assistance of D. Edgell. This research was supported by Medical Research Council of Canada Grant MT7137. J.-P. Praud is a scholar of the Fonds de la Recherche en Santé du Québec.

REFERENCES