Origins of the inhibiting effects of nasal CPAP on nonnutritive swallowing in newborn lambs

Nathalie Samson,1 Bianca Roy,1 Alain Ouimet,2 François Moreau-Bussière,1 Dominique Dorion,1,3 Sandeep Mayer,2 and Jean-Paul Praud1,3

1Équipe de Recherche Périmatale sur les Ovins, Departments of Pediatrics and Physiology, 2Department of Surgery, and 3ENT Division, Université de Sherbrooke, Sherbrooke, Quebec, Canada

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NASAL CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP) has been used in newborns for a number of years for treating respiratory distress syndrome and apneas of prematurity and as a bridge between endotracheal tube ventilation and spontaneous ventilation (4, 6, 10). However, laryngeal functions, such as nonnutritive swallowing (NNS), which fulfills the important task of clearing upper airways from secretions and liquids refluxed from the stomach, have not been specifically assessed in newborns during nasal CPAP, aside from one study wherein gotomy prevented this inhibition. However, application of CPAP on nonnutritive swallowing in newborn lambs.

MATERIALS AND METHODS

Animals

A total of 18 lambs (3 groups of 6 lambs) were included in the study. The study protocol was approved by the ethics committee of the Université de Sherbrooke for animal care and experimentation.

Common Surgical Preparation

Aseptic surgery was performed under general anesthesia (2% isoflurane, 30% N2O, 68% O2). Anesthesia was preceded by an intramuscular injection of ketamine (10 mg/kg), atropine sulfate (0.1 mg/kg), an intravenous bolus (10 ml/kg) of Ringer lactate solution, and one dose of ketoprofen (3 mg/kg intramuscularly) for analgesia, repeated if needed 12 h after the first dose. Antibiotics (5 mg/kg gentamicin and 0.05 ml/kg duplocilline) were administered intramuscularly before surgery and daily thereafter. Bipolar gold-plated stainless steel barbed broach electrodes were inserted into both thyroarytenoid muscles (TA) through the thyroid cartilage for electrical activity (electromyogram, EMG) recording. A bipolar electrode, custom-manufactured from two stainless steel Michel cutaneous staples (CDMV, St-Hyacinthe, QC, Canada), was inserted 5 cm distal to the esophageal inlet to record esophageal muscle EMG. Two needle electrodes (E7-12; Grass Instruments, Quincy, MA) were inserted into the parietal cortex directly through the skull for electroencephalogram (EEG) recording. A third needle electrode was also inserted under the scalp as a ground. Leads from each electrode were subcutaneously tunneled to exit on the back of the animal. Finally, a catheter was introduced into the artery of the lamb’s forepaw to monitor arterial blood gases and pH. On experimental days, just before recordings, two needle electrodes (F-E2; Grass Instruments) were inserted sub-
cutaneously near the right eye socket for electrooculogram (EOG) recording. Euthanasia was performed by an intravenous injection of 100 mg/kg pentobarbital sodium. Correct electrode positioning was systematically verified at autopsy.

**Specific Surgical Preparation**

**Tracheotomized group.** A tracheostomy was performed between the fifth and sixth tracheal rings in six lambs. An external tracheal tube was sewn and glued around the tracheostomy, leaving the tracheal lumen free of any instrumentation (8).

**Bivagotomy group.** A two-step, bilateral, intrathoracic vagotomy using video-assisted thoracic surgery (Tele Pack control unit no. 20043120-020) was performed in six other lambs. A rigid endoscope (Hopkins II; Karl Storz Endoscopy Canada, Mississauga, ON, Canada) and surgical instruments (no. 30340 FL, 30310 DBS; Karl Storz, Tuttinglen, Germany) were inserted into the right pleural space through two small parietal incisions. The thoracic portion of the right vagus nerve was first visualized, dissected, and isolated. The central bared portion of an enameled chrome wire electrode (0.12-mm diameter; Leico Industries, New York, NY) was positioned around the nerve, immediately caudal to the origin of the laryngeal recurrent nerve. The two free ends of the enameled chrome wire ultimately exited the pleural cavity and were anchored to the skin. A similar procedure was subsequently performed on the left side.

**Isolated upper airway group.** The remaining six lambs underwent a laryngotracheal separation to isolate the upper airways. The separation was performed directly under the larynx, just above the first tracheal ring. The caudal end of the larynx was sewn and glued to a 2-cm-long Dacron aortic prosthetic tube, whose extremity was sutured to a neck stoma. The rostral end of the trachea was sewn closed, and an external tracheal tube was installed around a tracheostomy, as described above for the tracheotomized group.

**Recording Equipment**

To obtain data from prolonged recordings in lambs under the least restraining conditions possible, we used our custom-designed radiotelemetry system, composed of eight channels for nasal flow, ECG, EOG, EEG, and EMG recordings (9). The raw EMG signals were rectified, integrated, and moving timeaveraged (100 ms). Qualitative variations of lung volume were monitored using respiratory inductance plethysmography (Respiritrace; NIMS, Miami Beach, FL). A custom-made nasal mask was installed on the lamb’s muzzle during nasal ventilation, in such a manner that it was able to open its mouth at will (19). During tracheal ventilation (tracheotomized group), a cuffed endotracheal tube was inserted through the external tracheostomy tube. Nasal and tracheal CPAP were administered using a Neopuff infant resuscitator mechanical ventilator (Fisher & Paykel Healthcare, Auckland, New Zealand) with heated (33°C), humidified air. Polysomnographic signals were recorded using Acknowledge software (version 3.7.3; Biopac Systems, Santa Barbara, CA). In addition, an observer was continuously present to note all events occurring during recordings.

**Study Design**

All lambs were housed with their mother, with the exception of lambs of the isolated upper airway group, because of specific care related to permanent tracheostomy breathing. Lambs from that group were placed in a Plexiglas chamber (1.2 m×1.2 m×1 m), where they could move as freely as possible (connected to the ventilator) and sleep in the position in which they felt most comfortable. The CPAP level administered in all lambs and in all experimental conditions was 6 cmH2O.

**Bronchopulmonary receptor studies.** TRACHEOTOMIZED GROUP. Three different experimental conditions (1 per day) were tested in a random order: 1) a control recording during which the lamb breathed through its nasal mask, without CPAP and with the tracheostomy tube closed (no CPAP); 2) a recording with CPAP administered through the nasal mask with the tracheostomy tube closed (nasal CPAP, nCPAP); and 3) a recording with CPAP administered through a cuffed tracheostomy tube (tracheal CPAP, tCPAP).

**BIVAGOTOMY GROUP.** Three different experimental conditions were tested, but only the first two conditions could be randomized: 1) a control recording during which the lamb breathed through its nasal mask, without CPAP and with intact vagi (no CPAP); 2) a recording with CPAP administered through the nasal mask with the tracheostomy tube closed (nasal CPAP, nCPAP); and 3) an experimental condition that was carried out on the last experimental day in which CPAP was administered through the nasal mask after thoracic bivagotomy (postvagotomy experiment). Thoracic bivagotomy was performed as follows. The free ends of each vagus nerve wire electrode were attached to an electrocauter (model 770; Electrosectilis, Briterich, CA). Trac-
NNS (at the transition from expiration to inspiration), ie-type NNS (at described: e-type NNS (preceded and followed by expiration), ei-type ratory phase preceding and following NNS. Four types of NNS were burst (propagated NNS). NNS were defined depending on the respi-

Arterial blood gases

Values are means ± SD. No CPAP, lambs breathed through a nasal mask without continuous positive airway pressure (CPAP); nCPAP, lambs breathed through a nasal mask during administration of CPAP; iCPAP, lambs breathed through a tracheostomy tube during administration of CPAP; prevago, lambs breathed through a nasal mask during administration of CPAP after bilateral vagotomy; CPAP 6 UA, lambs breathed through a tracheostomy tube during administration of CPAP on the upper airways only; CPAP 6 LA, lambs breathed through a tracheostomy tube during administration of CPAP on the lower airways only; RR, respiratory rate; Ti, inspiratory time; TE, expiratory time.

Table 2. Distribution of states of alertness for each experimental condition for all lambs
condition in the isolated upper airway group, in which only five lambs were studied.

No statistical differences were observed \( (P = 0.6) \) when NNS frequency during the no-CPAP condition in QS was compared among all three experimental groups (tracheotomized group, 45 ± 17 h\(^{-1}\); bivagotomy group, 38 ± 10 h\(^{-1}\); and isolated upper airway group, 43 ± 13 h\(^{-1}\)). These results were similar to our previous results \( (19) \) obtained under the same conditions but in lambs with intact airways \( (36 ± 14 \text{ h}^{-1}) \) (Fig. 2).

**Effect of Positive Airway Pressure on NNS Frequency**

The effects of CPAP on NNS frequency are reported in Table 3.

**Bronchopulmonary receptor studies.** **TRACHEOTOMIZED GROUP.** Compared with no CPAP, both nCPAP and tCPAP inhibited total and isolated NNS frequency during QS \( (P < 0.0001) \). However, only nCPAP \( (P = 0.001) \) inhibited bursts of NNS frequency.

**BIVAGOTOMY GROUP.** Compared with no CPAP, nCPAP with intact vagus nerves inhibited total and isolated NNS \( (P < 0.0001) \) and bursts of NNS \( (P = 0.01) \) during QS. This NNS inhibition was not observed after bilateral vagotomy \( (P \geq 0.4 \text{ vs. no CPAP}) \).

**Isolated upper airway receptor study.** **ISOLATED UPPER AIRWAY GROUP.** Compared with no CPAP, CPAP on either the upper or the lower airways inhibited total \( (P < 0.0007) \) and isolated \( (P < 0.01) \) NNS frequency during QS. On the other

### Table 3. Frequency of total NNS, isolated NNS, and NNS bursts for all three studies during quiet sleep

<table>
<thead>
<tr>
<th></th>
<th>Tracheotomized Group</th>
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<th>Bivagotomy Group</th>
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<th>Isolated Upper Airway Group</th>
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<tr>
<td></td>
<td>tCPAP</td>
<td>nCPAP</td>
<td>Postvago</td>
<td></td>
<td>CPAP 6 LA</td>
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<tr>
<td></td>
<td>No CPAP</td>
<td>Mean±SD</td>
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<td>vs. No CPAP</td>
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<td>vs. No CPAP</td>
<td>vs. nCPAP</td>
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<tr>
<td>Total NNS frequency, h(^{-1})</td>
<td>45±17</td>
<td>24±14</td>
<td>0.0001*</td>
<td>1</td>
<td>26±12</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Isolated NNS frequency, h(^{-1})</td>
<td>41±15</td>
<td>22±10</td>
<td>&lt;0.0001*</td>
<td>0.5</td>
<td>25±11</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Bursts of NNS frequency, h(^{-1})</td>
<td>4±4</td>
<td>2±4</td>
<td>0.1</td>
<td>0.4</td>
<td>1±1</td>
<td>0.001*</td>
</tr>
</tbody>
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|                      | No CPAP              | Mean±SD                | P                |                        | Mean±SD                     | P                      |
|                      |                      | vs. No CPAP            | vs. Prevago      |                        | vs. No CPAP                 | vs. nCPAP              |
| Total NNS frequency, h\(^{-1}\) | 38±10                | 38±9                   | 0.6              | <0.0001*               | 28±8                       | <0.0001*               |
| Isolated NNS frequency, h\(^{-1}\) | 37±10                | 38±10                  | 0.6              | <0.0001*               | 28±8                       | <0.0001               |
| Bursts of NNS frequency, h\(^{-1}\) | 0.8±1                | 0.5±0.9                | 0.4              | 0.05                   | 0±0                         | 0.01*                 |

|                      | No CPAP              | Mean±SD                | P                |                        | Mean±SD                     | P                      |
|                      |                      | vs. No CPAP            | vs. CPAP 6 UA    |                        | vs. No CPAP                 | vs. nCPAP              |
| Total NNS frequency, h\(^{-1}\) | 43±13                | 31±9                   | <0.0001*         | 0.09                   | 33±10                       | 0.0007*               |
| Isolated NNS frequency, h\(^{-1}\) | 39±9                 | 30±9                   | 0.0003*          | 0.04*                  | 33±10                       | 0.01*                 |
| Bursts of NNS frequency, h\(^{-1}\) | 3±5                  | 0.9±1.3                | 0.2              | 0.3                    | 0.4±0.7                     | 0.04*                 |

Values are means ± SD; \( n = 6 \) for each study with the exception of the tCPAP condition in the tracheotomized group and the CPAP 6 LA condition in the isolated upper airway group, in which \( n = 5 \). NNS, nonnutritive swallowing. *P values indicate statistically significant differences.

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hand, only the application of CPAP on the upper airways inhibited bursts of NNS frequency ($P = 0.04$).

In summary, CPAP consistently decreased total and isolated NNS frequency in QS when administered directly on the lower airways. This NNS inhibition was prevented by elimination of vagal afferent messages originating from bronchopulmonary receptors. In addition, application of CPAP on the upper airways only inhibited total, isolated, and bursts of NNS frequency during QS.

**NNS-Breathing Coordination**

The effects of CPAP on NNS-breathing coordination are reported in Table 4 and Fig. 3. Overall, NNS-breathing coordination was similar for all experimental conditions. While i-type NNS were the most frequent, e-type NNS were the least common. Furthermore, ei-type NNS were slightly more frequent than ie-type NNS in almost all experimental conditions, with the exception of the prevago condition of the bivagotomy group. Compared with no CPAP, both tCPAP (P = 0.009) and nCPAP (P = 0.003) decreased i-type NNS. Moreover, nCPAP 6 decreased ei-type NNS (P = 0.009). In contrast, tCPAP 6 increased e-type NNS (P = 0.008).

**DISCUSSION**

**Statement of Principal Findings**

The present study provides new insight on reflex mechanisms, which are involved in the inhibiting effects of nasal CPAP on spontaneous NNS in the neonatal period. Our results show that a CPAP of 6 cmH$_2$O inhibits NNS during QS when administered directly on the lower (subglottal) airways. This NNS inhibition is prevented when most vagal afferent messages, originating from bronchopulmonary receptors, are eliminated by bilateral vagotomy. In addition to the NNS-inhibiting effects of bronchopulmonary receptors, our results also reveal

![Table 4. Coordination between NNS and phases of the respiratory cycles](image)
that the application of a CPAP on the isolated upper airways inhibits NNS during QS. Finally, the present study reveals that CPAP does not alter NNS-breathing coordination in any of our animal models, all of which were uniquely designed using sophisticated surgical techniques.

**Effect of Positive Airway Pressure on NNS Frequency**

Nasal CPAP has been previously shown to inhibit water-induced swallowing in conscious adult humans (12) and spontaneous NNS in newborn lambs during QS (19). However, the precise reflex mechanism(s) by which swallowing is inhibited by nasal CPAP remains unclear. Data from the literature either suggest that the upper airways receptors or the bronchopulmonary receptors are implicated.

**Bronchopulmonary receptor studies.** Three types of afferent bronchopulmonary receptors are traditionally described, including the SARs, the rapidly adapting stretch receptors or "irritant" receptors, and the bronchopulmonary C-fiber endings (24). Since administration of CPAP is associated with lung inflation at a constant transmural pressure, rapidly adapting receptors and bronchopulmonary C-fibers are not stimulated by CPAP (2). Thus nasal CPAP could particularly inhibit NNS by stimulation of SARs by a vagally mediated lung reflex. In support of this hypothesis, continuous lung inflation brought about by continuous negative extrathoracic pressure in awake adult humans inhibits water-triggered swallows (7). The involvement of the SARs was deemed to be further supported by observing that voluntary hyperpnea (hypocapnic or normocapnic) inhibits water-induced swallows in awake adult humans (26). Moreover, our present results showing that a tracheal CPAP inhibits NNS in a manner similar to that of nasal CPAP suggest an involvement of bronchopulmonary receptors. This is further confirmed by our observation that bilateral vagotomy prevents NNS inhibition by nasal CPAP. The latter result also suggests that chest wall receptors are not implicated in NNS inhibition. In brief, the present data strongly suggest that the inhibiting effect of nasal CPAP on NNS during QS in the newborn lamb is mediated by a reflex mechanism originating from the bronchopulmonary receptors, most likely the SARs.

**Isolated upper airway group**

Nasal CPAP has been previously shown to inhibit water-induced swallowing in conscious adult humans (12) and spontaneous NNS in newborn lambs during QS (19). However, the precise reflex mechanism(s) by which swallowing is inhibited by nasal CPAP remains unclear. Data from the literature either suggest that the upper airways receptors or the bronchopulmonary receptors are implicated.

Theoretically, inhibition of NNS could be elicited by stimulation of upper airway receptors, including pressure, drive, cold (flow), irritant, and C-fiber receptors, all of which are particularly numerous in the nasal and laryngeal regions (20). The pressure receptors, whose activity is modulated by negative or positive pressure, account for most receptors of the nasal and laryngeal regions (20). Since the air driving CPAP in the present experiments was heated at body temperature and at a constant pressure, the inhibiting effect observed with CPAP on isolated upper airways is most probably mediated by stimulation of mechanoreceptors. Interestingly, direct application of CPAP on the isolated larynx was recently shown to enhance thyroarytenoid muscle activity in piglets (22). Results obtained in our isolated upper airway group show that, in this experimental condition, NNS inhibition is as important when CPAP is applied on the upper airways as on the lower airways. However, we do not have any satisfactory explanation for our observation that the inhibiting effect of CPAP on the upper airways appears less...
consistent among our various experimental conditions than the effect of CPAP on the lower airways. Overall, our results strongly suggest that bronchopulmonary receptors consistently mediate NNS inhibition by nasal CPAP, whereas upper airway receptors participate in the inhibition in certain experimental conditions only.

NNS-Breathing Coordination

NNS breathing-coordination is crucial for minimizing the risk of aspiration or prolonged apneas, especially in vulnerable infants, such as preterm newborns. Results of the present study confirm our previous results that inspiratory NNS are more frequent than expiratory NNS in control conditions (no CPAP) in newborn lambs (16, 19). These results also illustrate that, overall, application of CPAP has no systematic effect on NNS breathing-coordination in our three different animal models and experimental conditions. Interestingly, the absence of any alteration, after elimination of most bronchopulmonary afferent messages, suggests that they are not important for NNS-breathing coordination in neonates. We already showed that many conditions and external stimuli, such as prematurity (15), maturation and rumination (18), nasal ventilation (19), and hypoxia (3), do not alter NNS-breathing coordination in lambs. All these findings further support our hypothesis that NNS-breathing coordination is well established at the central nervous system level from birth.

Validation of Our Animal Models

According to a recent review, considerable gaps still exist in our knowledge on the modulation of upper airway muscles by bronchopulmonary afferents (2). With this in mind, we specifically developed two unique animal models to study the effects of upper airway vs. bronchopulmonary receptors on the regulation of NNS. First, we developed a chronically isolated upper airway lamb model. Results obtained in this model, when a CPAP was directly administered via a tracheostomy tube, reproduce results obtained in our tracheotomized group. This consistent finding in our two experimental groups suggests that upper airway surgery in our isolated upper airway group did not alter NNS activity in any manner, which would preclude any further conclusions. Furthermore, the relevance of this model for our physiological studies on NNS is suggested by the clinical observation that bottle feeding was identical to that in lambs with intact airways and that there were no signs of upper airway hypersecretion. Finally, NNS frequency in baseline conditions (no CPAP) was not different from that in lambs in the other experimental groups of the present study or in lambs having undergone no airway surgery in a previous study (19).

Second, we developed a lamb model using a two-step, intrathoracic bilateral vagotomy by using video-assisted thoracic surgery. This model offers several advantages over previously reported lamb models with bilateral vagotomy (14, 25). On the one hand, video-assisted thoracic surgery allows for a much less aggressive intervention than the use of a standard thoracotomy. In addition, the two-step procedure is better tolerated by the lamb, allowing investigators to wait for postoperative recovery during which the two vagal nerves still remain operational and to use each lamb as its own control. Again, the relevance of this bilateral vagotomy model was shown by the absence of observable swallowing abnormalities, both clinically during and between bottle feeding and when computing NNS frequency in baseline conditions.

Hence, the development of these two unique lamb models constitutes a very important aspect of the present study. It paves the way for further studies exploring the origins of various upper airway functions in the neonatal period, including, e.g., studies on the laryngeal chemoreflexes (23) or during nasal ventilation (11).

In conclusion, the present study illustrates for the first time that the inhibiting effect of nasal CPAP on NNS in newborn lambs during QS is mediated by stimulation of the bronchopulmonary receptors (most likely the SARs). Besides this consistent effect in all our experimental conditions, our results show that the inhibitory effect of CPAP also can be mediated by stimulation of upper airway receptors in certain conditions. The results also illustrate that, overall, the application of CPAP in newborn lambs has no systematic effect on NNS-breathing coordination, suggesting that NNS-breathing coordination is well established at the central nervous system level from birth. Finally, the present study has enabled the development of two unique and sophisticated animal models relevant for studying the modulation of upper airway muscles by bronchopulmonary afferents.

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