Effect of nasal continuous or intermittent positive airway pressure on nonnutritive swallowing in the newborn lamb

Nathalie Samson, Marie St-Hilaire, Elise Nsegbe, Philippe Reix, François Moreau-Bussière, and Jean-Paul Praud

Neonatal Respiratory Research Unit, Departments of Pediatrics and Physiology, University of Sherbrooke, Sherbrooke, Quebec, Canada

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The present study was aimed at investigating the effects of nasal continuous positive airway pressure (nCPAP; 6 cmH2O) or intermittent positive pressure ventilation (nIPPV; 10/4 cmH2O) on nonnutritive swallowing (NNS) and on the coordination between NNS and phases of the respiratory cycle, while taking into account the potential effects of states of alertness. Twelve full-term lambs were chronically instrumented at 48 h after birth for polysomnographic recordings, including NNS, diaphragm electromyographic activity, respiratory movements, pulse oximetry, and states of alertness. Studies in control conditions, with nCPAP and nIPPV, were performed in random order in nonsedated lambs at 4, 5, and 6 days of life. Results demonstrate that nCPAP significantly decreased overall NNS frequency, more specifically isolated NNS during quiet sleep and bursts of NNS in active sleep. In comparison, the effects of nIPPV on NNS frequency were more variable, with an inhibition of NNS only in wakefulness and an increase in isolated NNS frequency in active sleep. In addition, neither nCPAP nor nIPPV disrupted the coordination between NNS and phases of the respiratory cycle. In conclusion, nCPAP inhibits NNS occurrence in newborn lambs. Clinical relevance of this novel finding is related to the importance of NNS for clearing the upper airways from secretions and gastric content frequently regurgitated in the neonatal period.

A total of 12 mixed-bred lambs were involved in the study. All lambs were born at term by spontaneous vaginal delivery at our local provider’s farm. The protocol of the study was approved by the ethics committee for animal care and experimentation of the University of Sherbrooke.

Surgical Preparation

Aseptic surgery was performed on the 2nd day of life under general anesthesia (2% isoflurane-30% N2O-68% O2). Anesthesia was preceded by an intramuscular injection of atropine sulfate (0.1 mg/kg) and ketamine (10 mg/kg). Antibiotics (5 mg/kg gentamicin and 50 mg/kg ampicillin) were administered intramuscularly before surgery and daily until the end of the experiments. One dose of ketoprofen (3 mg/kg intramuscularly) was systematically given immediately after induction of the anesthesia for analgesia and repeated if needed the next day. Chronic instrumentation included placement of bipolar enameled chrome wire electrodes (diameter 0.1 mm) in both thyroarytenoid (TA); a glottal constrictor) and diaphragm muscles for electromyographic (EMG) activity recording. Custom-made electrodes for electroencephalogram (EEG) and electrocardiogram (ECG) were placed as previously described. Two platinum needle electrodes (F-E2, Grass Instrument, Quincy, MA) were placed into the trachea for pulse oximetry, and states of alertness. Studies in control conditions, with nCPAP and nIPPV, were performed in random order in nonsedated lambs at 4, 5, and 6 days of life. Results demonstrate that nCPAP significantly decreased overall NNS frequency, more specifically isolated NNS during quiet sleep and bursts of NNS in active sleep. In comparison, the effects of nIPPV on NNS frequency were more variable, with an inhibition of NNS only in wakefulness and an increase in isolated NNS frequency in active sleep. In addition, neither nCPAP nor nIPPV disrupted the coordination between NNS and phases of the respiratory cycle. In conclusion, nCPAP inhibits NNS occurrence in newborn lambs. Clinical relevance of this novel finding is related to the importance of NNS for clearing the upper airways from secretions and gastric content frequently regurgitated in the neonatal period.

While swallowing and respiratory problems are among the most frequent disorders encountered in neonates, the interrelationships between both of these functions are not completely known. This is especially true for nonnutritive swallowing (NNS), which fulfills the important function of clearing upper airways from secretions and liquids refluxed from the stomach. The latter is particularly relevant to the neonatal period, when immaturity of the lower esophageal sphincter is responsible for gastroesophageal reflux in virtually all infants, exposing them to apneas, bradycardias, and oxyhemoglobin desaturation via the laryngeal chemoreflexes and/or tracheal aspiration. Recent studies in infants (4) and in full-term and preterm lambs (17, 19) have provided new information on NNS in basal conditions, including the effects of states of alertness and the links with apneas. However, conditions in the neonatal intensive care units are frequently far from “basal,” and many conditions (e.g., tachypnea) or therapeutic interventions (e.g., nasal application of airway pressure may interfere with NNS and its coordination with breathing. The present study focuses on the potential effects of nasal continuous positive airway pressure (nCPAP; CPAP) and intermittent positive pressure ventilation (IPPV) on NNS in the neonatal period for two reasons. First, previous results in awake human adults suggest that nCPAP inhibits water-induced swallowing (13). To our knowledge, these are the only available results on the effects of nasal application of positive airway pressure on NNS in the medical literature. Second, nCPAP and nasal IPPV (nIPPV) are increasingly being used in newborns for treating apneas of prematurity (2, 11) and respiratory distress syndrome (16, 23, 24), as well as for reducing the rate of extubation failure following endotracheal mechanical ventilation (3, 5). The aim of the present study conducted in newborn lambs was, therefore, to 1) test the hypothesis that nCPAP and nIPPV decrease NNS frequency; 2) examine whether nCPAP and/or nIPPV influences the coordination between NNS and phases of the respiratory cycle; and 3) assess the potential effects of states of alertness on the above relationships.

MATERIALS AND METHODS

Animals

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Surgical Preparation

Aseptic surgery was performed on the 2nd day of life under general anesthesia (2% isoflurane-30% N2O-68% O2). Anesthesia was preceded by an intramuscular injection of atropine sulfate (0.1 mg/kg) and ketamine (10 mg/kg). Antibiotics (5 mg/kg gentamicin and 50 mg/kg ampicillin) were administered intramuscularly before surgery and daily until the end of the experiments. One dose of ketoprofen (3 mg/kg intramuscularly) was systematically given immediately after induction of the anesthesia for analgesia and repeated if needed the next day. Chronic instrumentation included placement of bipolar enameled chrome wire electrodes (diameter 0.1 mm) in both thyroarytenoid (TA; a glottal constrictor) and diaphragm muscles for electromyographic (EMG) activity recording (7). Custom-made electrodes for electroencephalogram (EEG) and electrocardiogram (ECG) were placed as previously described. Two platinum needle electrodes (F-E2, Grass Instrument, Quincy, MA) were placed into the trachea for pulse oximetry, and states of alertness. Studies in control conditions, with nCPAP and nIPPV, were performed in random order in nonsedated lambs at 4, 5, and 6 days of life. Results demonstrate that nCPAP significantly decreased overall NNS frequency, more specifically isolated NNS during quiet sleep and bursts of NNS in active sleep. In comparison, the effects of nIPPV on NNS frequency were more variable, with an inhibition of NNS only in wakefulness and an increase in isolated NNS frequency in active sleep. In addition, neither nCPAP nor nIPPV disrupted the coordination between NNS and phases of the respiratory cycle. In conclusion, nCPAP inhibits NNS occurrence in newborn lambs. Clinical relevance of this novel finding is related to the importance of NNS for clearing the upper airways from secretions and gastric content frequently regurgitated in the neonatal period.

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outer lower region and the inner upper region of the right eye socket for electrooculogram (EOG) recording. Finally, a catheter was introduced in the artery of the forepaw of each lamb, to collect arterial blood samples for analyses of pH, arterial PO$_2$, arterial PCO$_2$, and HCO$_3^-$ concentration. This catheter was left in place for the entire duration of the study and flushed daily with heparin solution. Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. Correct electrode positioning was systematically verified at autopsy.

**Measurement Apparatus**

To obtain data from prolonged recordings (with periods of wakefulness and sleep) in lambs, we used our recently developed and specifically designed radiotelemetry system (10). The transmitter used in this study was composed of eight differential channels for nasal flow, ECG, EOG, EEG and four EMG recordings. The eight transmitted signals were fed from the receiver to the acquisition system. The raw EMG signals were rectified, integrated, and averaged (moving time average of 100 ms). Constant acquisition of the arterial O$_2$ saturation was possible with the use of a pulse oximeter transmitting its signals also by a specific radiotelemetry system (18). Qualitative assessment of thoracic and abdominal movements and of lung volume variations was monitored in all lambs with the use of respiratory inductance plethysmography (Respitrace, NIMS, Miami Beach, FL). A custom-made nasal mask (Fig. 1) was built to fit the muzzle of each lamb as follows. First, we made a mould of the lamb muzzle with the use of an aluminum padded, malleable splint (Sam-splint, CDMV, St-Hyacinthe, QC). This mould was modified to allow the fastening of a double nasal cannula (4.5 endotracheal tube cut and filled) and of a nasogastric tube (8 Fr). The mould was then covered by strips of plaster to obtain a solid shell. Once dried, the splint was removed from the shell, which was attached behind the lamb’s head with a cotton ribbon. The mask was then filled with dental paste (Examix no. 138006, Servident, St-Laurent, QC) to ensure nasal ventilation without air leakage. Of note, the nasal mask did not impede mouth opening, in an attempt not to interfere with NNS. Nasal ventilation was performed by using a Siemens Servo 300 mechanical ventilator with humidified air. Finally, the CPAP/IPPV values were monitored continuously, using a calibrated pressure transducer (MP 45-30-871, Validyne, Northridge, CA) connected to the inspiratory circuit of the ventilator. An observer was always present with the lamb to note all events occurring during the recordings. Polysomnographic signals were recorded on a personal computer (Pentium 3) using a computerized acquisition device (MP150, Biopac Systems, Santa Barbara, CA) and software (Acknowledgement 3.7.3, Biopac Systems). Collected data were stored on compact disk for further analysis.

**Design of the Study**

On arrival in our animal quarters, the lambs were cared for without their mother. They were fed ad libitum three times a day with ewe’s milk at 8:00 AM and 1:00 and 6:00 PM and thrived normally. Surgical instrumentation was performed on the 2nd day of life. Polysomnographic recordings of 4–6 h were performed on 3 consecutive days at 4, 5 and 6 days of life, between 2:00 and 8:00 PM. Lights were off throughout the recordings. Each lamb was studied without sedation, while placed in a transport cage (80 × 90 × 80 cm), where it could move freely and sleep in the position it felt more comfortable, which was variable from one lamb to another. On experimental days, the lamb was fed freely at 8:00 AM, received only 100 ml at 1:00 PM (to prevent accumulation of milk in the stomach, which could prevent evacuation of air by the nasogastric tube), and was fed freely at the end of the polysomnographic recordings. Three different ventilatory modes were tested in random order using the nasal mask + nasogastric tube, including no cmH$_2$O CPAP (control), 6-cmH$_2$O CPAP (nCPAP 6), and 10/4-cmH$_2$O IPPV (nIPPV 10/4). An observer was always present with the lamb during the polysomnographic recordings and continuously noting the lamb’s behavior. This helps determination of the states of alertness, which was performed posteriorly.

**Data Analysis**

All signals were carefully observed and analyzed throughout the recordings in relation to the states of alertness. Standard electrophysiological and behavioral criteria were used to define wakefulness (W), quiet sleep (QS), active sleep (AS), and arousal, as previously described (20). NNS activity was recognized by a brief, high-amplitude TA EMG burst, as previously validated (17). NNS activity was then characterized either as an isolated NNS, or a burst of NNS, which was defined for the purpose of this study as two or more NNS occurring within a period of 4 s (>90% of NNS bursts were comprised within a 4-s time period). In a first analysis, NNS frequency (number of NNS per hour) was calculated for isolated NNS, bursts of NNS, and total NNS (isolated + bursts of NNS), for each state of alertness and in each ventilatory mode. NNS occurring during active W was not analyzed because of motion artifacts, which prevented us from analyzing TA EMG signal. Thus calculation of NNS frequency during W was restricted to periods of quiet W. Second, diaphragmatic EMG and the sum signal of the respiratory inductance plethysmography were used to recognize the phase of the respiratory cycle disrupted by isolated NNS. Each isolated NNS activity was then defined, depending on the respiratory phase preceding and following NNS. Four types of isolated NNS were described (17): e-type NNS (preceded and followed by an expiration), ei-type NNS (at the transition from expiration to inspiration), i-type NNS (preceded and followed by an inspiration) and ie-type NNS (at the transition from inspiration to expiration).
Fig. 2. A: spontaneous and isolated nonnutritive swallowing (NNS) [inspiration (i)-type NNS] occurring during quiet sleep (QS). B: burst of NNS occurring during active sleep (AS). 

Results

General Results

Animals. Twelve lambs weighing 4.2 kg (SD 0.9) (range 3.1–6.1) on the day of surgery were included in the study. One lamb was ultimately excluded because it did not sleep during the recordings. Analyses were, therefore, carried out in 11 lambs in control conditions, 10 of 11 with nCPAP 6, and 10 of 11 with nIPPV 10/4 (technical problems being responsible for the absence of one lamb in the last two groups).

Blood-gas analyses. No statistical differences were observed between arterial blood-gas parameters taken before installation of the nasal mask and at the end of the experiment, regardless of nasal pressure applied. Values before and after recordings were, respectively, pH = 7.47 (SD 0.03) and 7.46 (SD 0.02), arterial Po2 = 72 (SD 10) and 76 Torr (SD 9), arterial Pco2 = 35 (SD 3) and 35 Torr (SD 5), and HCO3 = 26 (SD 3) and 25 mM (SD 4).

States of alertness. Duration of polysomnographic recordings was 141.4 h, with a mean recording time of 4.2 h (SD 0.7) (range 2.2–6.3) for each experimental condition in each lamb. Mean duration of states of alertness in each lamb was 2.4 h (SD 0.7) (range 1.6–3.5) in W, 1.3 h (SD 0.4) (range 0.4–2.4) in QS, and 0.1 h (SD 0.1) (range 0–0.5) in AS. Ventilatory modes did not affect the distribution of the states of alertness (P values ranging from 0.5 to 0.9). The three states of alertness were present in all but two lambs, for which no AS was observed during the control recording. Sample tracings obtained in a lamb with nCPAP 6 are given in Fig. 2. While NNS sometimes occurred simultaneously with an EEG arousal, as illustrated in Fig. 2, this was not a frequent finding. Indeed, 14% (12% in control conditions; 13% with nCPAP 6; 16% with nIPPV 10/4) and 4% (8% in control conditions; 2% with nCPAP 6; 3% with nIPPV 10/4) of all NNS occurred with an EEG arousal during QS and AS, respectively.

NNS Frequency in Control Conditions

The effects of the states of alertness on NNS frequency in control conditions are illustrated in Fig. 3B. First, total NNS frequency was significantly higher in AS [174 h−1 (SD 72); range 50–302] and quiet W [63 h−1 (SD 22); range 33–108] than in QS [36 h−1 (SD 14); range 12–58]. In addition, total NNS frequency was significantly higher in AS than in quiet W. Similarly, isolated NNS frequency was significantly higher in AS [88 h−1 (SD 25); range 50–114] and quiet W [58 h−1 (SD 15); range 33–87] than in QS [35 h−1 (SD 15); range 12–58]. Isolated NNS frequency was also significantly higher in AS than quiet W. Finally, the same picture was seen for NNS bursts, which occurred significantly more often during AS [32 h−1 (SD 20)] and quiet W [2 h−1 (SD 3)] than during QS [0.3 h−1 (SD 0.5)]. In addition, NNS bursts were significantly more frequent in AS than in quiet W. All P values for the above analyses were ≤0.0004.

Effects of Nasal Application of Positive Airway Pressure on NNS Frequency

In all cases, lambs with nCPAP 6 or nIPPV 10/4 exhibited similar patterns in NNS frequency with regard to their distribution among the three states of alertness, compared with that observed in lambs in control conditions (Fig. 3B).

Total NNS. Results are illustrated in Fig. 3, A and B, top. Compared with control conditions, nCPAP 6 inhibited NNS in 5 of 10 lambs during quiet W, 9 of 10 lambs during QS, and 3 of 8 lambs during AS (P = 0.03, QS vs. quiet W). In addition, when all three states of alertness were grouped together, nCPAP 6 decreased overall NNS frequency (P = 0.02). This
decrease in NNS was statistically significant during QS ($P < 0.0001$), but not during quiet W ($P = 0.07$) or AS ($P = 0.2$). Meanwhile, nIPPV 10/4 inhibited NNS in 6 of 10 lambs during quiet W, 8 of 10 lambs during QS, and 4 of 8 lambs during AS, compared with control conditions. However, the magnitude of this inhibition was lower than that observed with nCPAP 6 and was not statistically significant when all states of alertness were grouped together ($P = 0.8$). On the other hand, this decrease in total NNS by nIPPV 10/4 did reach borderline significance during quiet W ($P = 0.05$), but not during AS ($P = 0.7$) and QS ($P = 0.5$). Overall, nCPAP 6 had significantly greater inhibiting effects on total NNS than nIPPV 10/4 during QS ($P = 0.0002$). No statistical difference was noted between nCPAP 6 and nIPPV 10/4 for both quiet W ($P = 1.0$) and AS ($P = 0.2$).

Isolated NNS. Results are illustrated in Fig. 3, A and B, middle. Compared with control conditions [51 h$^{-1}$ (SD 14)], nCPAP 6 [37 h$^{-1}$ (SD 10)] decreased isolated NNS frequency ($P = 0.0008$), when all states of alertness were grouped together. As in total NNS, this decrease in isolated NNS frequency was statistically significant during QS ($P < 0.0001$), but not during quiet W ($P = 0.1$) or AS ($P = 0.6$). Conversely, nIPPV 10/4 [43 h$^{-1}$ (SD 14)] did not significantly decrease isolated NNS frequency, compared with control conditions, when combining all states of alertness ($P = 0.06$). Further analyses revealed that nIPPV 10/4 decreased isolated NNS frequency during quiet W ($P = 0.05$, i.e., borderline significance) but increased their frequency during AS ($P = 0.04$). No significant effect was observed during QS ($P = 0.5$). Overall, the inhibiting effects of nCPAP 6 on isolated NNS were significantly greater than those of nIPPV 10/4 during QS ($P = 0.0003$). No statistical difference was noted between nCPAP 6 and nIPPV 10/4 for both quiet W ($P = 1.0$) and AS ($P = 0.2$).

Bursts of NNS. Results are illustrated in Fig. 3, A and B, bottom. Compared with control conditions [3 h$^{-1}$ (SD 3)], nCPAP 6 [2 h$^{-1}$ (SD 2)] did not decrease NNS burst frequency, when all states of alertness were grouped together ($P = 0.8$). However, further analysis revealed that nCPAP 6 significantly decreased NNS burst frequency during quiet W ($P = 0.01$) but not during quiet W ($P = 0.9$) or QS ($P = 0.7$). By contrast, nIPPV 10/4 [1 h$^{-1}$ (SD 1)] decreased NNS burst frequency relative to control conditions [3 h$^{-1}$ (SD 3)], when all states of alertness were grouped together ($P = 0.003$). A significant decrease in NNS burst frequency by nIPPV was observed during quiet W ($P = 0.02$), but not during AS ($P = 0.2$) or QS ($P = 0.9$). Moreover, the inhibiting effects of nIPPV 10/4 on NNS burst frequency were statistically greater than those of nCPAP 6, when all states of alertness were grouped together ($P < 0.0001$) and during quiet W ($P = 0.0005$). Identical results were obtained when comparing the effects of

Fig. 3. Relationship between NNS frequency (number of NNS per hour) and the different ventilatory conditions studied [no CPAP (Cont.), 6-cmH$_2$O nasal CPAP (nCPAP) (6), 10/4-cmH$_2$O nasal intermittent positive pressure ventilation (nIPPV) (10/4)] when all states of alertness are taken together (A) and each state of alertness is considered individually (B). Top: results for total NNS frequency; middle: results for isolated NNS frequency; bottom: results for NNS burst frequency. Please note that a statistical difference is present among each state of alertness within each ventilatory mode, except between wakefulness (W) and QS for NNS burst during nIPPV 10/4. Also, please note the different scale for the y-axis. Results are presented as means (SD). *$P < 0.05$. 

Isolated NNS. Results are illustrated in Fig. 3, A and B, middle. Compared with control conditions [51 h$^{-1}$ (SD 14)], nCPAP 6 [37 h$^{-1}$ (SD 10)] decreased isolated NNS frequency ($P = 0.0008$), when all states of alertness were grouped together. As in total NNS, this decrease in isolated NNS frequency was statistically significant during QS ($P < 0.0001$), but not during quiet W ($P = 0.1$) or AS ($P = 0.6$). Conversely, nIPPV 10/4 [43 h$^{-1}$ (SD 14)] did not significantly decrease isolated NNS frequency, compared with control conditions, when combining all states of alertness ($P = 0.06$). Further analyses revealed that nIPPV 10/4 decreased isolated NNS frequency during quiet W ($P = 0.05$, i.e., borderline significance) but increased their frequency during AS ($P = 0.04$). No significant effect was observed during QS ($P = 0.5$). Overall, the inhibiting effects of nCPAP 6 on isolated NNS were significantly greater than those of nIPPV 10/4 during QS ($P = 0.0003$). No statistical difference was noted between nCPAP 6 and nIPPV 10/4 for both quiet W ($P = 1.0$) and AS ($P = 0.2$).
nCPAP 6 and nIPPV 10/4 on the average number of NNS within a burst.

In summary, both the states of alertness and the application of positive airway pressure influenced NNS frequency. The main effects can be summarized as follows. First, the highest NNS frequency was consistently observed in AS, and the lowest one in QS, whatever the experimental condition and the type of NNS (total, isolated, or in bursts). Second, the overall effect of nCPAP 6 was inhibitory on NNS and was observed in QS for isolated NNS and in AS for bursts of NNS. Third, the effect of nIPPV 10/4 on NNS frequency was more variable, being an overall decrease in NNS in quiet W, but an increase in isolated NNS in AS.

Coordination Between NNS and Phases of the Respiratory Cycle

Coordination between NNS and phases of the respiratory cycle could be analyzed for 92% of all NNS. Results are illustrated in Fig. 4.

Control conditions. During quiet W in control conditions, i-type NNS [26 h⁻¹ (SD 14), 43%] and ei-type NNS [20 h⁻¹ (SD 10), 32%] were the most frequent. Both of these NNS types were significantly more frequent than the ie-type [11 h⁻¹ (SD 5), 19%]. Finally, e-type NNS [4 h⁻¹ (SD 3), 8%] were the least common, significantly less than the three other NNS types. This pattern of NNS respiration coordination was similar for both QS and AS as well. All of the above P values were <0.002.

nCPAP 6. Overall, during the three states of alertness, the same pattern of NNS respiration coordination was observed with nCPAP 6 than in control conditions. The only difference was an absence of statistical difference with nCPAP 6 between ie-type and e-type NNS during AS. In addition, a statistically significant decrease in ei-type NNS was noted with nCPAP 6 during quiet W (P = 0.006). Similarly, a statistically significant decrease in i-type NNS (P = 0.04), ei-type and ie-type NNS (P < 0.0001), and e-type NNS (P = 0.03) was noted with nCPAP 6 during QS. Finally, only the ie-type NNS (P = 0.02) exhibited a statistically significant decrease with nCPAP 6 during AS.

nIPPV 10/4. Overall, during the three states of alertness, the pattern of NNS respiration coordination was largely similar to that observed for both control conditions and nCPAP 6, with, again, an absence of statistical difference with nIPPV 10/4 between ie-type and NNS during AS. Further analyses showed a statistically significant decrease in i-type NNS (P = 0.02) between control conditions and nIPPV 10/4 during quiet W. Conversely, compared with no CPAP, the ei-type NNS were significantly increased during nIPPV 10/4 during AS (P = 0.03); the latter condition was the only exception in the pattern of NNS respiration coordination, with the ei-type tending to be more frequent than the i-type.

In summary, overall, nCPAP 6 and nIPPV 10/4 had virtually no effect on the coordination between NNS and phases of the respiratory cycle. The increase in the ei-type with nIPPV 10/4 appeared as an exception.

DISCUSSION

Statement of Principal Findings

The present study brings new knowledge on the effect of application of positive airway pressure via a nasal mask on spontaneous NNS activity in the neonatal period. First, the results provided herein confirm that nCPAP significantly decreases NNS frequency and show that the sleep states have a modulating effect, with inhibition of isolated NNS during QS and of bursts of NNS in AS. Second, unique results on nIPPV show that its effects on NNS frequency are more variable, with an inhibition of NNS only in quiet W and an increase in isolated NNS frequency in AS. Third, the present results also demonstrate that nasal positive airway pressure does not alter the coordination between NNS and phases of the respiratory cycle. Relevance of the present results mainly stems from the importance of NNS for clearing airway secretions and gastric content regurgitated from the stomach, which contributes to the prevention of apnea, bradycardia, and hypoxemia events in the newborn.
Inhibition of NNS Frequency by Nasal Application of Positive Pressure

Although a previous study reported an inhibition of water-induced swallows by nCPAP in conscious adult humans (13), studies documenting the effects of nCPAP or nIPPV on spontaneous NNS are currently nonexistent. Thus the present observation in the newborn lamb that nCPAP 6 inhibits NNS frequency more than nIPPV 10/4 during sleep is a significant finding. However, the mechanisms responsible for NNS inhibition during nasal application of positive airway pressure remain unclear. A decrease in upper airway secretions by a drying effect of positive pressure application is unlikely, due to the use of humidified air. In addition, this drying effect would likely be more prominent with nIPPV 10/4 than with nCPAP 6, due to greater convection effects with nIPPV, hence leading to a stronger NNS inhibition than with nCPAP, which is contrary to the present results, at least in sleep. Other hypotheses also come to mind. First, inhibition of the swallowing reflex could be elicited by the stimulation of pressure receptors located in the upper airways, especially in the laryngeal mucosa. Thus the upper airway receptors would be stimulated by nCPAP, but there is no evidence that this could affect swallowing frequency. These receptors, whose activity is modulated by the application of negative or positive pressure in the upper airways, account for the majority (~60%) of receptors described in the laryngeal region (12, 21). Stimulation of positive-pressure receptors of the laryngeal region by application of nasal ventilatory support could, therefore, trigger inhibitory afferent messages relayed to the swallowing centers in the brain stem. Recent studies have shown that direct application of continuous or intermittent positive pressure on the isolated piglet larynx modulates the activity of laryngeal muscles. Interestingly, the effects on laryngeal muscle activity were more important with continuous than with intermittent application of positive pressure, similar to our present observations (22). Further studies assessing the effects of positive pressure applied via a tracheostomy in spontaneously breathing lambs, and therefore without stimulation of upper airway receptors, should enable verification of this hypothesis. Second, NNS inhibition could be mediated by stimulation of slow-adapting bronchopulmonary receptors, since the administration of nasal ventilatory support is associated with lung inflation. Stimulation of these receptors by nasal ventilation would thereby inhibit swallowing by a vagally mediated lung reflex. In support of this hypothesis, continuous lung inflation brought about by application of continuous negative extrathoracic pressure in awake adult humans significantly inhibits water-triggered swallowing activity (9). In addition, voluntary hyperpnea (hypocapnic or normocapnic) was shown to inhibit water-induced swallowing in awake adult humans (25). This hypothesis will need further testing by repeating the present study in vagotomized lambs. Finally, given that NNS appear to be triggered when piriform sinuses are full (15), distension of the latter by application of positive pressure in the upper airways could delay filling and decrease NNS frequency. Further studies by applying CPAP through a tracheostomy should allow testing of this hypothesis. Regardless of the mechanism(s) involved, it is clear that nCPAP 6, and to a lesser degree nIPPV 10/4, inhibit NNS in the newborn lamb.

Coordination Between NNS and Phases of the Respiratory Cycle

A perfect coordination between swallowing and respiratory activity is necessary to minimize the risk of aspiration or prolonged apneas, especially in vulnerable infants, such as preterm newborns. Our laboratory has previously reported and discussed the differences regarding that coordination in lambs, compared with previous results in newborn and adult humans and in adult mammals (17, 19). The present study illustrates that, overall, nasal application of positive-pressure ventilation in newborn lambs does not modify the coordination between NNS and phases of the respiratory cycle, i.e., that the i-type NNS is the most frequent, and the e-type NNS the least frequent. This observation may seem somewhat surprising in newborn lambs, given previous observations of altered coordination between swallowing and phases of the respiratory cycle in adult humans in various experimental conditions, including imposed hypercapnia (14), respiratory elastic loading (8), lung inflation due to negative extrathoracic pressure (9), and voluntary hyperpnea (25). Although we cannot provide any clear explanations for what might be considered as discrepant results, important differences between experimental conditions in lambs vs. adults should be emphasized, however, including experimental maneuvers used to impose respiratory changes, the presence of full consciousness throughout the experiments in humans (and thus awareness of the maneuvers), and the study of water-triggered swallowing in humans vs. spontaneous NNS in lambs. Finally, species differences, including body position (erect/sitting vs. on all fours/supine) cannot be ruled out.

Absence of Effects of Nasal Mask and/or Nasogastric Tube on NNS

Given preliminary personal observations that nasal application of positive pressure ventilation in newborn lambs led to important gastric distension, the use of a nasogastric tube in the present experiments was deemed mandatory, although there were concerns that the use of a nasal mask and/or a nasogastric tube might alter NNS. Comparison of present observations with nasal mask + nasogastric tube, but without CPAP (i.e., what we call control conditions herein), with previous results obtained in our laboratory in lambs of the same age, but without mask or nasogastric tube, reveal similar results, notably with regard to NNS frequency, NNS respiratory coordination, and the effects of sleep states (17).

Clinical Relevance of Present Findings

In addition to the physiological knowledge gained from the present study, the data also have clear and relevant clinical implications, especially with regard to preterm newborn infants, who at times must spend numerous weeks on nCPAP for treatment of recurrent apneas. Indeed, the observed decrease in NNS frequency may have deleterious effects in newborn infants under nCPAP treatment, by an increased risk of aspirating saliva or regurgitated gastric content. In addition, the inhibitory effect on NNS may impact on swallowing matura-

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translate into prolonged abnormalities of swallowing activity. However, the virtual absence of alterations in the coordination between NNS and phases of the respiratory cycle is somewhat reassuring. In addition, the absence of deleterious effects of nasal mask and nasogastric tube, especially considering that the latter is used in all preterm infants born before 35 wk of gestational age, is of significant clinical relevance.

In conclusion, the present study illustrates for the first time that NNS is inhibited by nasal application of positive airway pressure in newborn lambs, especially by CPAP 6 during sleep and IPPV 10/4 during quiet W. Although this finding could have important clinical implications for maturation of swallowing activity, elucidation of the mechanisms responsible for this inhibition awaits further study.

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REFERENCES