Nonnutritive swallowing and respiration relationships in preterm lambs

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Submitted 20 January 2004; accepted in final form 16 May 2004

Reix, Philippe, Julie Arsenault, Carole Langlois, Théophile Niyonsenga, and Jean-Paul Praud. Nonnutritive swallowing and respiration relationships in preterm lambs. J Appl Physiol 97: 1283–1290, 2004.—The aim of the present study was to assess the effects of the different states of alertness on 1) nonnutritive swallowing (NNS) frequency, 2) the relationship between NNS and the respiratory cycle, and 3) the association of NNS with spontaneous apneas. Recordings of sleep states, diaphragm and laryngeal constrictor electrical activity, nasal flow, electrocardiogram, respiratory inductance plethysmography, and pulse oximetry were obtained from six preterm lambs without sedation. Analysis of 2,468 NNS showed that 1) NNS frequency was higher during quiet wakefulness and active sleep (AS) than in quiet sleep; 2) in all states of alertness, a greater number of NNS (38%) were preceded and followed by an inspiration; 3) although NNS and central apneas were rarely coincidental, AS appeared to favor their association; and 4) most obstructive apneas occurred in AS and were coincidental with bursts of NNS. Compared with results in full-term lambs, premature birth does not modify the NNS-respiratory coordination. However, AS in preterm lambs is characterized by a higher association of NNS bursts with obstructive apneas.

Swallowing is a fundamental function, starting as early as 11 wk of gestation in humans and readily efficient at 35 wk of gestation (6). Prenatally, in utero swallowing is an important element in several functions, including regulation of amniotic fluid volume and composition, ingestion and potential recirculation of solutes from the fetal environment, and maturation of the gastrointestinal tract (32). Postnatally, efficient nutritive swallowing activity is crucial for sufficient food intake and normal growth. However, the newborn must now coordinate swallowing with air breathing. Feeding problems due to immature swallowing function and problems of coordination with breathing are among the most frequent problems in preterm newborns (1, 33). In addition to nutritive swallows, nonnutritive swallows (NNS) are equally of major importance for clearing both airway secretions and liquid regurgitated from the stomach (10, 36). Impairment of either nutritive swallowing or NNS activity can lead to acute life-threatening events.

Only a few studies have specifically addressed NNS in newborn mammals, which include data on their frequency, on the fine coordination between NNS and phases of the respiratory cycle, and on the potential association between bursts of NNS and apneas, all of which, unfortunately, raise several discrepancies. First, data on NNS frequency are conflicting. Whereas NNS frequency was reported to be higher in active sleep (AS) than during both wakefulness (W) and quiet sleep (QS) in both term and preterm human newborns (10), as well as in full-term lambs (28), a third recent study has found no effects of sleep states in full-term human newborns (4). Second, regarding the precise coordination between NNS and respiratory phases, NNS were reported to occur randomly within the respiratory cycle during sleep in preterm humans (37). In opposition, our laboratory has recently shown that NNS frequently occurs during inspiration in full-term newborn lambs (28), regardless of the state of alertness. Third, obstructive and mixed apneas and, to a lesser extent, central apneas were reported to be frequently associated with NNS in preterm infants (20, 21). However, the potential influence of the states of alertness on this association was not assessed.

Henceforth, the aim of the present study was to assess, in preterm newborn lambs, the influence of the states of alertness on 1) the frequency of both isolated NNS and bursts of NNS, 2) the coordination between NNS and respiration, and 3) the association between NNS and spontaneous apneas. Moreover, the present study gave us the opportunity to compare the new data obtained in preterm lambs with those recently published in full-term lambs (28). It was hypothesized that NNS frequency is higher in AS than in QS, that states of alertness do not modify the coordination between NNS and phases of the respiratory cycle, and that bursts of NNS are linked to obstructive apneas, especially in AS.

Materials and Methods

Experiments were conducted in six preterm lambs with a postconceptual age of 132 days (normal gestation: 147 days) and a mean birth weight of 3.1 ± 0.2 kg (range: 2.9–3.6 kg). The protocol of the study was approved by the Ethics Committee for Animal Care and Experimentation of our institution.

Preterm Lamb Model

Four lambs were delivered vaginally after prenatal lung maturation, as previously described (29). Two lambs were delivered by cesarean section under epidural anesthesia with 5 ml of 2% lidocaine. Exogenous surfactant (10 ml of bovine lipid extract surfactant; BLES, London, ON) was given to the lamb by transcervical, intratracheal injection immediately after birth and repeated 24 h later. Standard care for the first postnatal hours was implemented as previously reported, including continuous positive airway pressure for a few hours and O2 supplementation as needed (27). Continuous nasogastric feeding with natural ewe’s milk was initiated 3–4 h after birth and replaced by discontinuous gastric feeding after 1–2 days. The nasogastric tube was systematically removed for polysomnographic recordings.

Surgical Preparation

Surgery was performed 2–3 days after birth under general anesthesia (1–2% isoflurane + 30% N2O + 68% O2). Atropine sulfate (150
µg/kg subcutaneously) was given preoperatively with 5 mg/kg ketamine and 100 µg/kg midazolam intramuscularly. Bipolar enameled chrome wire electrodes were inserted into the thyroarytenoid (TA) and diaphragm muscles for recording electromyographic (EMG) activity (11), together with custom-made electrodes for electroencephalogram (EEG), electrooculogram (EOG), and ECG recordings (29). Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. The lambs were also enrolled in another study (27), which required a tracheostomy. The latter was performed as previously described, with the tracheostomy tube being entirely external to the tracheal lumen (27). Postoperative care included intra-
preoperatively with the tracheostomy tube being entirely ex-
ternal to the tracheal lumen (27). Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. The lambs were also enrolled in another study (27), which required a tracheostomy. The latter was performed as previously described, with the tracheostomy tube being entirely external to the tracheal lumen (27). Postoperative care included intra- muscular buprenorphine (50 µg/kg, one dose at the end of surgery) as well as intramuscular injection of 50 mg/kg ampicillin and 2.5 mg/kg gentamicin daily thereafter. Lambs were euthanized at the end of experiments by an overdose of pentobarbital. Correct electrode positioning was systematically verified at autopsy.

Recording Equipment

Polysomnographic recordings were obtained by using our custom-made radiotelemetry system, as previously described (14). The raw EMG signals were rectified, integrated, and moving-time averaged (100 ms). Nasal airflow was recorded by using a thermocouple. Thoracic and abdominal volume variations were assessed with their sum by using respiratory inductance plethysmography (Respirtrac, NIMS, Miami Beach, FL). Oxygenation was continuously monitored by using a pulse oximeter built from a Nonin OEM with transmission by radiotelemetry (27). The oximeter probe was attached at the base of the tail. All signals were recorded on a Power Macintosh 7300 by using the Acknowledge 3.2 acquisition software (Biopac Systems, Santa Barbara, CA).

Design of the Study

Each lamb was studied without sedation, at least 48 h after surgery. The telemetry transmitters were connected to the electrode leads and attached to the lamb’s back before each recording session. Because telemetry transmission was not yet available for respiratory inductance plethysmography, lambs were studied in an incubator for 3–6 h daily with no restraints. Periods of feeding, including 5 min following feeding, were excluded from analysis. NNS frequency and coordination with respiration analysis were performed during the first postnatal week in the six lambs, similar to our previous study on NNS published in full-term lambs (28). In addition, the association of NNS to apneas was also studied during the second postnatal week in four of the preterm lambs, because most apneas occurred during this period. The tracheostomy was kept tightly closed during recordings.

Data Analysis

States of alertness. Standard electrophysiological and behavioral criteria were used to define W, QS, and AS, from EEG, EOG, and continuous visual observation (29). Arousal from QS was characterized by sudden disappearance of high-amplitude waves in the EEG, whereas arousal from AS was recognized by direct observation of the lamb and disappearance of intense EOG activity.

NNS activity. NNS was recognized by a brief, high-amplitude TA EMG burst with interruption of nasal airflow, as previously validated (28). Analysis of NNS from TA EMG signal was then pursued as follows: NNS frequency (number of NNS per hour) was first calculated for each state of alertness in each lamb and then averaged for the six lambs as a whole. Periods of active W were excluded from analysis, because of the high proportion of movement artifacts. Thus calculation of NNS frequency during W was restricted to periods of quiet W. Second, recognition of the phase of the respiratory cycle disrupted by isolated NNS was performed by visual analysis of the traces of diaphragmatic EMG, nasal airflow, and TA EMG activity, as previously validated (28), together with the respiratory inductance plethysmography signal. As previously reported, four types of NNS were described, including e-type NNS (preceded by and followed by expiration), ei-type NNS (at the transition from expiration to inspiration), ie-type NNS (at the transition from inspiration to expiration), and i-type NNS (preceded by and followed by inspiration) (28) (Fig. 1). NNS frequency was then calculated for each type of NNS in each state of alertness and averaged for each lamb and then for the six lambs as a whole. The frequency of NNS bursts, defined as two or more NNS occurring within a 10-s period (28), was then calculated for each state of alertness. We also classified NNS bursts according to the number of NNS by bursts (2, 2–4, 4–6, and >6 NNS by period of 10 s), based on the previous observation that high-frequency NNS bursts would occur more frequently during AS, as reported in the fetal lamb (32). Finally, the association of NNS bursts with apneas was assessed as follows: for this purpose, apneas defined as a cessation of nasal flow for at least 3 s were considered. Central, obstructive, and mixed apneas were recognized from the diaphragmatic EMG activity and the respiratory efforts observed on the respiratory inductance plethysmography traces. The apnea index was first calculated for each apnea type in each state of alertness and averaged for each lamb and then averaged for the six lambs as a whole. In addition, the average minimal arterial O2 saturation from pulse oximetry value and the average percentage of decrease in heart rate were calculated. Then the percentage of NNS bursts associated with each type of apnea, including isolated central apneas, central apneas during periodic breathing, and obstructive and mixed apneas, was calculated. Also, in an attempt to assess whether NNS triggered apneas, the percentage of apneas immediately (≤1 s) preceded by NNS was calculated for each state of alertness.

Statistical analysis. Statistical analyses on NNS frequency and the relationships between NNS and the respiratory cycles were performed by using SAS and SPSS software packages, as previously described (28). For quantitative variables, summary results were expressed as means ± SD. The effects of the state of alertness on NNS frequency, respiratory-swallowing coordination, and the association of NNS with apneas were tested by using generalized linear models with repeated measures (GEMROD procedure of SAS). The working correlation structure chosen was the exchangeable type. For count data, generalized linear models consisted of Poisson regression models. For continuous data, linear models, which assume normality for the response variables, were used. Normality was tested first (Shapiro-Wilks test); when absent, square root transformation of the response variable was performed. Results with P values ≤0.05 were considered significant, and the Bonferroni correction was used whenever applicable.

RESULTS

Total duration of recordings in the six lambs during the first postnatal week was 41 h, with a mean total recording time of 153 ± 68 min (range 68–223 min) for quiet W, 111 ± 32 min (range 71–155 min) for QS, and 64 ± 32 min (24–109 min) for AS. A total of 2,468 NNS were recorded during the first postnatal week in our six preterm lambs, including 1,627 isolated NNS and 841 NNS distributed in 297 bursts.

NNS Frequency

States of alertness influenced the frequency of both isolated NNS and NNS bursts. Isolated NNS frequency was significantly higher in both W (57 ± 10 h−1; range 46–75 h−1) and AS (66 ± 26 h−1; range 39–113 h−1) than during QS (37 ± 5 h−1; range 28–42 h−1; P < 0.0001) (Fig. 2A). Similarly, NNS burst frequency was significantly higher in AS (31 ± 22 h−1; range 13–66 h−1) and W (8 ± 6 h−1; range 2–19 h−1) than in QS (3 ± 3 h−1; range 0–8 h−1; P < 0.0001) (Fig. 2B). Although there was no periodicity in the swallowing process,
NNS were found to occur more regularly in QS than in AS. Moreover, no EEG arousals were observed simultaneous to NNS.

Relationship Between NNS Activity and Respiratory Phases

Of 1,627 isolated NNS recorded, a total of 1,440 isolated NNS were analyzed. Percentages of analyzed NNS over recorded NNS were distributed as follow in the three states of alertness: 83% (703/850) during quiet W, 93% (335/361) during QS, and 97% (402/416) during AS. As a whole, i-type NNS were the most frequent type of NNS (19/1100611 h11; 44%). NNS occurring at the transition of respiratory phases (ei- and ie-type) were equally distributed [13 ± 10 h−1 (22%) and 13 ± 6 h−1(28%), respectively]. Finally, the frequency of e-type NNS (3 ± 3.5 h−1; 6%) was the lowest of all, significantly lower than the i-type (P = 0.0001), the ei-type (P = 0.0004), and the ie-type (P = 0.0001). States of alertness had little effect on this pattern of distribution. Although the ei-type NNS tended to be more frequent than the e-type NNS in both W and AS, this was not statistically significant (P = 0.007 and 0.025, respectively, P < 0.004 expected using the Bonferroni correction). The effect of the states of alertness was significant only for the ie-type NNS, which were more frequent in AS than in QS (P = 0.0006) (Fig. 3).

Bursts of NNS

A total of 841 NNS were distributed in 297 bursts. Most NNS bursts (239/297 = 79%) had two to four NNS per burst, 19% (56/297) had four to six NNS per burst, and only 2% (5/297) had more than six NNS per burst. In the two latter groups, 54% of the bursts occurred during AS, 39% during W, and 7% during QS. Overall, the mean number of NNS by burst was 2.8 ± 1.3, and there were no statistical differences among states of alertness (P = 0.9). Finally, the duration of NNS bursts was longer during W and AS (5.2 ± 2.6 and 5.2 ± 2.5 s, respectively) than in QS (3.3 ± 1.5 s) (P = 0.001).
Association Between NNS and Spontaneous Apneas

A total of 2,196 apneas were recorded during the first 2 postnatal wk in four lambs. Most apneas (n = 2,119) were central apneas, including 943 isolated apneas in four lambs and 1,176 apneas during periodic breathing epochs in two lambs. Only 77 apneas were obstructive or mixed. The index of isolated central apneas was 15.4 ± 8.5 h⁻¹ during W, 20.6 ± 12.6 h⁻¹ during QS, and 11.9 ± 5.8 h⁻¹ during AS. Periodic breathing epochs were almost exclusively observed during QS (never during AS). The overall index of obstructive and mixed apneas was 2.6 ± 2.3 h⁻¹, with 79% of these apneas occurring in AS, 12% in QS, and 9% in W. On average, duration of obstructive and mixed apneas was shorter (5.3 ± 3.0 s) than duration of central apneas (7.0 ± 2.6 s) (P = 0.012). Minimal arterial O₂ saturation from pulse oximetry was lower after obstructive and mixed apneas (86 ± 7%) than after central apneas (90 ± 5%) (P = 0.0002). However, the percentage of decrease in heart rate was not statistically different between obstructive and mixed apneas and central apneas (17 ± 14 vs. 23 ± 17%; P = 0.09).

Whereas the association between NNS and apneas was highly variable from one lamb to another, it was not a close association, especially for central apneas. Overall, with central and mixed and obstructive apneas being considered altogether, 95% of NNS were not associated with apneas, and 90% of apneas occurred without NNS. The association was dependent, however, on the state of consciousness and was clearly different between central and mixed and obstructive apneas. Indeed, few central apneas, either isolated or within periodic breathing, were associated with NNS in quiet W (11 ± 8% of apneas, range 1–18%) and QS (9 ± 4% of apneas, range 6–14%). In comparison, the association between NNS and central apneas was more frequent during AS (28 ± 16%, range 14–50%) (P < 0.05). On the other hand, 74 ± 10% (range 59–82%) of mixed and obstructive apneas were associated with NNS, with this association being significantly higher than for central apneas (P = 0.0001). When present, NNS were usually observed within the first 3 s after apnea onset, and only 0.4% of the apneas were immediately preceded by NNS. Moreover, the average number of NNS during apneas was not correlated with apnea duration (r = 0.04, P = 0.2 for central apneas; r = 0.03, P = 0.14 for obstructive and mixed apneas). Finally, assessment of the effects of states of alertness revealed that, in AS, NNS frequency was higher during apneas compared with periods without apneas, whether they were obstructive or mixed (P < 0.0001) or central (P < 0.02). This was not the case for apneas in quiet W (P = 0.9) and QS (P = 0.5). Also, NNS frequency outside apneas was higher in AS than in quiet W and QS (P < 0.03).

The distribution of isolated NNS vs. bursts of NNS was also different, depending on apnea type. Indeed, most NNS occur-
ring during central apnea were found to be single NNS, i.e., 74% of NNS observed during isolated central apneas and 100% of NNS observed during periodic breathing. Conversely, 68% of the NNS observed during obstructive and mixed apneas occurred in bursts \( P = 0.009 \) (Fig. 4A). Moreover, NNS bursts were found to be more often associated with isolated central apneas in AS than in QS \( P = 0.014 \) or W \( P = 0.013 \) (Fig. 4B) (episodes of periodic breathing were excluded from this analysis, for there were no NNS bursts during periodic breathing). Examples of NNS bursts associated with an obstructive apnea and a central apnea are shown in Fig. 5.

**DISCUSSION**

The present study brings new light on the interaction between NNS and respiration in immature, preterm lambs. Results show that 1) states of alertness influence the frequency of both isolated NNS and bursts of NNS and have a modest influence on the respiratory-NNS coordination; 2) preterm lambs have a higher rate of NNS bursts than full-term lambs; 3) whereas NNS and central apneas are rarely coincidental, AS appears to favor their association; and 4) most obstructive apneas occur in AS and are coincidental with bursts of NNS.

**Frequency of NNS in the Preterm Lamb**

**Influence of rapid eye movement sleep.** Both isolated NNS and bursts of NNS were found to be more frequent in AS. Several studies have consistently documented the same effect of rapid eye movement (REM) sleep on NNS frequency, including perinatally and in adult life (15). Indeed, NNS frequency was found to be higher in AS in near-term ovine fetuses (review in Ref. 32), in full-term newborn lambs (12, 28), and in both full-term and preterm healthy infants (10, 36).

Although a recent study did not find any effect of sleep states on NNS frequency in human newborns, this may be due to the study design, whereby swallows were measured while saline was slowly infused into the pharynx (4). The influence of REM sleep in triggering irregular and higher respiratory rate is an established fact (review in Ref. 31). The same is true for vegetative functions such as heart rate (16). Given the close localization and similar organization of the central pattern generators driving these functions (9), it is not surprising that REM sleep exerts a similar influence on NNS, respiration, and heart rate.

**Influence of premature birth.** In our study, although frequency of isolated NNS was similar to what our laboratory previously observed during sleep in full-term lambs (28), isolated NNS frequency was, in fact, lower during W in preterm than full-term lambs. This apparent discrepancy must be interpreted carefully. Indeed, it should be underlined that, whereas preterm lambs in the present study were recorded while permanently lying in an incubator, the full-term lambs were recorded remotely while moving freely in a pen with their mother and siblings. Consequently, compared with full-term lambs, recordings in preterm lambs were characterized by a higher proportion of quiet W, which may be responsible for the differences between preterm and full-term lambs during W.

Our study also revealed that frequency of NNS bursts was significantly higher in preterm than in full-term lambs, in all three states of alertness (3- to 4-fold in W and AS and 25-fold in QS, respectively). A plausible explanation could be that preterm lambs have increased sensitivity to fluid stimuli compared with full-term lambs. Accordingly, pharyngeal stimulation with warm water was shown to yield more frequent responses (swallowing, obstructed breath, apnea) in preterm than in full-term infants (24, 25). Moreover, multiple swallows were commonly observed in preterm infants and considered to be maladaptive, because a single swallow may be sufficient for clearing the airway (25). Of note, as in full-term lambs, the frequency of NNS bursts was much higher in AS than QS. Our hypothesis is that NNS activity is part of the REM sleep-related phasic motor activity, equivalent to pontogeniculocipital waves, bursts of rapid eye movements, or body twitches (8).

**Coordination Between Isolated NNS and the Respiratory Cycle**

Our results indicate that, regardless of sleep stage, 72% of NNS are preceded by inspiration in preterm lambs. This is in
agreement with our laboratory’s recent results in full-term lambs (28) but not with a number of previous data in adult humans. Indeed, in awake adult humans, 75–95% of swallows, mostly induced by water (range: 1–100 ml), were consistently reported to begin in expiration (2, 7, 13, 17, 19, 23, 26, 34). By contrast, 55% of NNS began during inspiration in awake adult goats (5). Moreover, whereas breathing resumed most often with expiration in adult humans (7, 13, 17, 26, 34), it resumed most often with inspiration in adult goats (5). While species differences (ruminants vs. humans) may explain that NNS-respiration coordination (coordination in preterm and full-term lambs is similar to that in adult goats but different from adult humans), a number of alternative explanations are possible, including awareness of the study and induction of swallows by water in awake humans and immaturity. To our knowledge, only two studies of the precise coordination between NNS and respiration have been performed in the neonatal period. Whereas spontaneous NNS have been reported to occur randomly within the ventilatory cycle in sleeping infants (37), our laboratory recently reported that 57% of NNS begin in inspiration in full-term lambs (28). The latter results are in accordance with results of the present study, with an even higher frequency in preterm lambs (72 vs. 57%). The higher frequency of NNS beginning in inspiration in awake adult goats (5) and both full-term (28) and preterm (present study) lambs suggests that the precise coordination between respiration and NNS is mature at birth, even in the preterm lamb.

Our results suggest that the states of alertness have only a slight incidence on the coordination between NNS and respiration in preterm lambs, i.e., a modest but significant decrease of the i-type NNS in QS compared with AS. This is identical to what we have recently reported in full-term lambs (28). The reason for this effect of QS is unclear but may reflect the stronger inspiratory drive present in QS compared with AS. This is in accordance with the observation that neonatal central apneas are more frequent in AS than QS (30). To our knowledge, there are no other data on the effects of states of alertness on the coordination between respiration and NNS.

Fig. 5. A: spontaneous obstructive apnea associated with NNS burst (4 NNS in 2.6 s; the fifth NNS was not taken into account because it occurred after resumption of breathing) in a 9-day-old preterm lamb (141 days of postconceptional age) during AS. B: NNS burst (5 NNS in 2.7 s; the fifth NNS occurred simultaneously to breathing resumption) associated with central apnea occurring during an AS epoch in the same lamb. SpO2, arterial oxygen saturation from pulse oximetry; Pleth, plethysmographic pulse waveform.
NNS and Spontaneous Neonatal Apneas

The potential mechanisms explaining the association between apneas and NNS in AS certainly warrant further discussion. On the one hand, central mechanisms cannot explain all of the present observations. First, as apnea onset consistently occurs before NNS, the association cannot be explained by sustained inhibition of brain stem respiratory neurons due to repetitive activity of the swallowing central pattern generator (35). Second, although it has been previously suggested that desinhibition of the swallowing central pattern generator during apneas could increase swallowing frequency (21), this would not explain our results that show that NNS bursts are more frequent during obstructive and mixed apneas compared with central apneas. Third, the potential effect of chemical stimuli, such as hypoxia and/or hypercapnia secondary to apnea, in triggering NNS burst is unlikely. This is supported by stimuli, such as hypoxia and/or hypercapnia secondary to central apneas. Third, the potential effect of chemical stimuli, such as hypoxia and/or hypercapnia secondary to obstructive and mixed apneas and NNS (20). Some discrepancies between the present findings and previous findings, such as the absence of any effect related to the apnea type (4), may be due to the design of the study and/or age of the infants studied.

Conclusion

Regardless of the state of alertness, the precise coupling between NNS and the different phases of the respiratory cycle do not appear to be influenced by premature birth in nonseparated, obstructive and mixed apneas are frequently associated with NNS bursts. Further studies are necessary to investigate the mechanisms controlling the coordination between NNS and respiration and to explain the association between isolated NNS and central apneas in QS, and between NNS bursts and obstructive and mixed or central apneas in AS.

ACKNOWLEDGMENTS

The authors thank Christophe Grenier and Christine Mayrand-Charrette for technical assistance. The authors also acknowledge the generous donation of surfactant by BLES, London, ON, Canada.

GRANTS

The research was supported by Canadian Institutes of Health Research Grant MOP15558, and the Quebec Foundation for Research into Children’s Diseases. P. Reix is a scholar of the Société de Pneumologie de Langue Française. J.-P. Praud is a National Researcher of the Fonds de la Recherche en Santé du Québec.

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