Effects of hypoxia and hypercapnia on nonnutritive swallowing in newborn lambs

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1Neonatal Respiratory Research Unit, Departments of Pediatrics and Physiology, 2Department of Mechanical Engineering, Université de Sherbrooke, Sherbrooke, Quebec, Canada; 3Institut National de la Santé et de la Recherche Médicale, U642; 4Laboratoire Traitement du Signal et de l’Image, Université de Rennes 1; and 5Département de Médecine de l’Enfant et de l’Adolescent, Néonatologie, Centre Hospitalier Universitaire Rennes, Rennes, France

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Duvareille C, Lafrance M, Samson N, St-Hilaire M, Pladys P, Micheau P, Bournival V, Langlois C, Praud J-P. Effects of hypoxia and hypercapnia on nonnutritive swallowing in newborn lambs. J Appl Physiol 103: 1180–1188, 2007. First published July 12, 2007; doi:10.1152/japplphysiol.00318.2007.—The aim of the present study was to investigate the effects of hypoxia and hypercapnia on NNS-breathing coordination in newborn lambs, while taking into account the potential effects of states of alertness. Six lambs were chronically instrumented for recording NNS-breathing coordination, in full-term (34) and preterm lambs (33). NNS and respiration share a common aerodigestive tract (the upper airways), and their central pattern generator (CPG) is located at the same medullary site. It is thus conceivable that any adverse condition, such as hypoxia or hypercapnia, that alters breathing in the newborn may influence NNS frequency and the precise NNS-breathing coordination and, consequently, promote lung aspiration and/or respiratory instability. In addition, postnatal maturation of swallowing and NNS-breathing coordination may be altered by these conditions because of the high neural plasticity at this time. Thus the aim of the present study was to investigate the effects of hypoxia and hypercapnia, as well as hypoxia, on spontaneous NNS frequency and NNS-breathing coordination in newborn lambs by testing the hypothesis that these conditions alter NNS frequency in the three states of alertness and modify NNS-breathing coordination.

MATERIALS AND METHODS

Animals

Six mixed-breed lambs, born at full term by spontaneous vaginal delivery, were studied. The study protocol was approved by the 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 (1–2% isoflurane + 30% NO2 + 68% O2). Atropine sulfate (150 μg/kg im) was given preoperatively with ketamine (10 mg/kg) and midazolam (100 μg/kg). Antibiotics [5 mg/kg gentamicin and 7,500 IU/kg procaine penicillin-benzathine penicillin (Duplocillin)] were administered intramuscularly before surgery and daily thereafter until the end of the experiment. One dose of ketoprofen (3 mg/kg im) was systematically given immediately after induction of anesthesia for analgesia and repeated if needed on the next day. Chronic instrumentation was performed as previously described (43) and included bipolar electrodes into the thyroarytenoid (TA) muscle (a glottal adductor) and the diaphragm (Dia) for recording electromyographic (EMG) activity (TA and Dia EMG), two needle electrodes into the parietal cortex for electrocorticogram (ECOg), and two subcutaneous needle electrodes into the forelegs for electrocardiogram (ECG). An arterial catheter was introduced into the brachial artery for measurement of blood gases. Correct electrode positioning was systematically verified at autopsy.

Recording Equipment

The instrumentation was completed immediately before the recording session. Two needle electrodes were inserted subcutaneously near the right eye for electrooculogram (EOG) recording. Nasal airflow...
was recorded using a type J thermocouple (0.002-s response time), and respiratory thoracoabdominal movements were monitored with respiratory inductance plethysmography. Inspiratory and expiratory fractions of O$_2$ and CO$_2$ were continuously monitored using a nasal cannula and a Capnomac II (Datex-Ohmeda, Mississauga, ON, Canada). A radiotelemetry system (18) was used to continuously transmit signals of nasal flow, ECG, EOG, ECoG, and EMG. The raw EMG signals were rectified, integrated, and averaged (100-ms moving time). All signals were recorded on a personal computer (Fig. 1) using the MP100A data acquisition system and Acknowledge 3.7.3 software (Biopac Systems, Santa Barbara, CA).

**Design of the Study**

Lambs were studied without sedation 48 h after surgery (4 ± 1 days of age, 3.9 ± 0.5 kg body wt) between 6:00 and 11:30 AM. The lambs were housed with their mothers between all experiments. All recordings began ≥40 min after their last feeding. Each lamb underwent three recordings on three subsequent days while in a Plexiglas chamber (1.2 m$^3$) through which medical air, 21% O$_2$ + 5% CO$_2$ [hypercapnia (Hc)], or 10% O$_2$ + 0% CO$_2$ [hypoxia (Hx)] continuously flowed (12 l/min). Consistency of the gas mixture in the chamber was monitored throughout the recordings using an oximeter and a capnometer (models O$_2$ FC-1B and CO$_2$ CA-1B, Sable Systems, Las Vegas, NV). Each day corresponded to a different gas mixture, administered in random order. The chamber temperature (24°C) and humidity (70%) were maintained constant throughout the recordings. Lambs were monitored throughout the three recordings, and an observer was always present in the laboratory to note all events. In wakefulness (W), the lamb was standing or lying prone, with its head up; in quiet sleep (QS), the lamb was lying prone, usually with its head placed backwards on the shoulder; in active sleep (AS), the lamb was lying prone with its head and neck extended on the floor.

Three additional lambs were studied after addition of holes for hermetic gloves in two opposite walls of the chamber to enable manipulation of the lambs throughout the experiment without opening the chamber. The lambs were studied in the same three experimental conditions for assessment of variations in blood gases, saliva secretion, and body temperature. Saliva production was measured using a cotton ball inserted on a hairpin that was weighed (Gram Precision SL-50, Mississauga, ON, Canada) before and after insertion in the lamb’s mouth between the lower gums and the left cheek; the cotton ball inserted on a hairpin that was weighed (Gram Precision SL-50, Mississauga, ON, Canada) before and after insertion in the chamber. The lambs were studied in the same three experimental conditions (W, QS, and AS) without sedation 48 h after surgery (4 ± 1 days of age, 3.9 ± 0.5 kg body wt) between 6:00 and 11:30 AM. The lambs were housed with their mothers between all experiments. All recordings began ≥40 min after their last feeding. Each lamb underwent three recordings on three subsequent days while in a Plexiglas chamber (1.2 m$^3$) through which medical air, 21% O$_2$ + 5% CO$_2$ [hypercapnia (Hc)], or 10% O$_2$ + 0% CO$_2$ [hypoxia (Hx)] continuously flowed (12 l/min). Consistency of the gas mixture in the chamber was monitored throughout the recordings using an oximeter and a capnometer (models O$_2$ FC-1B and CO$_2$ CA-1B, Sable Systems, Las Vegas, NV). Each day corresponded to a different gas mixture, administered in random order. The chamber temperature (24°C) and humidity (70%) were maintained constant throughout the recordings. Lambs were monitored throughout the three recordings, and an observer was always present in the laboratory to note all events. In wakefulness (W), the lamb was standing or lying prone, with its head up; in quiet sleep (QS), the lamb was lying prone, usually with its head placed backwards on the shoulder; in active sleep (AS), the lamb was lying prone with its head and neck extended on the floor.

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**Data Analysis**

Standard electrophysiological and behavioral criteria were used to define W, QS, and AS from ECoG and EOG traces, together with careful, continuous observation of the lambs (35). Arousal from QS was characterized by sudden disappearance of high-amplitude waves in the ECoG for ≥3 s, and arousal from AS was recognized by direct observation of the lamb and disappearance of intense EOG activity. The percentage of time spent in each state of alertness, the mean duration and frequency of W, QS, and AS epochs, and the arousal frequency were calculated. Respiratory rate (RR) and heart rate (HR) were calculated for all epochs of W, QS, and AS recorded in all lambs when ≥60 s were spent in that epoch. Apneas were defined as two “missed” breaths compared with the two preceding respiratory cycles. Sighs were defined as an at least twofold increase in the amplitude of Dia EMG compared with the three preceding respiratory cycles. Apnea and sigh frequency were calculated in the three states of alertness.

As previously described, NNS activity was identified by a brief, large-amplitude TA EMG burst with interruption of nasal airflow (32). Average NNS was determined by calculation of total NNS (comprising isolated NNS and NNS occurring in bursts) frequency for each lamb in each state and in each gas condition (38). Calculation of NNS frequency in W was restricted to periods of quiet W. Dia EMG, plethysmographic, and nasal flow signals were used to identify four types of NNS from the respiratory phase preceding and following the NNS (34); e-type (preceded by and followed by expiration), ei-type (at the transition from expiration to inspiration), ie-type (at the transition from inspiration to expiration), and i-type (preceded by and followed by inspiration).

The effect of NNS type on respiratory timing was assessed on the breaths before (n + 1), and after (n + 1) the swallow and compared during air breathing, Hx, and Hc. For each isolated NNS, alterations in inspiratory time (Ti), expiratory time (Te), total breathing duration (Ttot), and duty cycle (Ti/Ttot) were measured. All values of respiratory timing were expressed as a percentage of the control value measured on the n − 3 breath for each NNS type.

All results were first averaged for each individual lamb and then for the entire group as a whole for each gas mixture.

**Statistical Analyses**

Statistical analyses were performed using SAS software, as previously described (34). Normality was first tested for all variables using the Shapiro-Wilks test. Summary results are expressed as means ±
gas condition, as shown by results obtained in the first and last
blood gases were constant throughout the recordings in each
Hc (4.1
Table 2. RR, HR, apnea, and sigh frequency in air, Hc, and Hx breathing

<table>
<thead>
<tr>
<th></th>
<th>Hc</th>
<th>P</th>
<th>Hx</th>
<th>P vs. Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>% W</td>
<td>44±8</td>
<td>53±9</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% QS</td>
<td>43±4</td>
<td>38±4</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% AS</td>
<td>12±4</td>
<td>9±5</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Epoch duration, s</td>
<td>120±32</td>
<td>180±77</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>%Q S</td>
<td>11±3</td>
<td>10±3</td>
<td>0.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>AS</td>
<td>4±2</td>
<td>3±2</td>
<td>0.04</td>
<td>0.7</td>
</tr>
<tr>
<td>Arousal frequency, h−1</td>
<td>22±7</td>
<td>21±9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>AS</td>
<td>30±10</td>
<td>42±27</td>
<td>0.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

n = 6. W, wakefulness; QS, quiet sleep; AS, active sleep; Hc, hypercapnia; Hx, hypoxia. P < 0.05 indicates statistically significant difference.

The mean effect of Hx or Hc on sleep architecture, NNS frequency and NNS-breathing coordination, RR and HR, apnea, and sigh frequency was estimated using generalized estimating equation models, which take into account the correlated nature of repeated measures (GENMOD procedure). The working correlation structure was the exchangeable type. For data that followed a normal distribution (HR and RR), no transformations were made. For the remaining data, generalized linear models consisted of Poisson regression models. P values were obtained using the z-statistics, a robust estimate of the standard error. Analyses of the relationship between respiratory rate and ie-type NNS percentage were performed using simple linear regression analysis. P < 0.05 was considered significant.

RESULTS

Total duration of polysomnographic recordings in six lambs was 24.4 h in air (mean ± SD per lamb: 4.1 ± 0.2 h), 24.6 h in Hc (4.1 ± 0.5 h), and 25.1 h in Hx (4.2 ± 0.6). Arterial blood gases were constant throughout the recordings in each gas condition, as shown by results obtained in the first and last hours of the recordings: arterial PO2 (Pao2) = 86 ± 21 and 82 ± 15 Torr, arterial PCO2 (Paco2) = 38 ± 0 and 38 ± 2 Torr, and pH = 7.47 ± 0.01 and 7.47 ± 0.002, respectively, in air; Pao2 = 105 ± 1 and 99 ± 1 Torr, Paco2 = 44 ± 0 and 43 ± 3 Torr, and pH = 7.40 ± 0.01 and 7.44 ± 0.04, respectively, in Hc; and Pao2 = 26 ± 4 and 26 ± 4 Torr, Paco2 = 25 ± 0 and 28 ± 1 Torr, and pH = 7.57 ± 0.01 and 7.55 ± 0.00, respectively, in Hx. Measurements of basal metabolism obtained in the three lambs showed a decrease in O2 consumption in Hx (10.5 ± 0 ml·min−1·kg−1) compared with air (15 ± 3 ml·min−1·kg−1) and Hc (17 ± 5 ml·min−1·kg−1).

Sleep Architecture

Data for sleep architecture are shown in Fig. 2 and Table 1. AS was achieved in all lambs in the three gas conditions. Overall, although Hc increased %W (i.e., time spent in W, expressed as a percentage of the total recording time), Hx had no significant effect on %W. During sleep, although %QS

Table 2. RR, HR, apnea, and sigh frequency in air, Hc, and Hx

<table>
<thead>
<tr>
<th></th>
<th>Hc</th>
<th>P</th>
<th>Hx</th>
<th>P vs. Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR, min−1</td>
<td>63±21</td>
<td>90±30</td>
<td>&lt;0.0001</td>
<td>0.3</td>
</tr>
<tr>
<td>QS</td>
<td>60±22</td>
<td>89±29</td>
<td>0.0002</td>
<td>0.3</td>
</tr>
<tr>
<td>AS</td>
<td>61±10</td>
<td>77±17</td>
<td>&lt;0.0001</td>
<td>0.9</td>
</tr>
<tr>
<td>HR, min−1</td>
<td>186±41</td>
<td>203±44</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>AS</td>
<td>188±41</td>
<td>202±44</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Apnea frequency, h−1</td>
<td>30±19.3</td>
<td>8±9.9</td>
<td>0.002</td>
<td>0.0008</td>
</tr>
<tr>
<td>AS</td>
<td>6±5.8</td>
<td>1±2.0</td>
<td>0.004</td>
<td>0.006</td>
</tr>
<tr>
<td>Sigh frequency, h−1</td>
<td>44±15.7</td>
<td>25±3.9</td>
<td>0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

n = 6. RR, respiratory rate; HR, heart rate. P < 0.05 indicates statistically significant difference.
decreased in Hc and increased in Hx, this was not related to a change in arousal frequency. Neither Hc nor Hx had a significant effect on %AS. Mean duration of W epochs was increased during Hc, but not Hx. Neither Hc nor Hx had a significant effect on the mean duration and frequency of QS and AS epochs.

Cardiorespiratory Parameters

Data for cardiorespiratory parameters are reported in Table 2. Regardless of the state of alertness, RR was increased in Hc and Hx, with no difference between Hc and Hx. Most apneas were postsigh apneas. A decrease in apnea and sigh frequency was observed in Hc during W and AS. Regardless of the state of alertness, Hx did not alter apnea or sigh frequency. Although Hx induced an increase in HR in all states of alertness, the increase during Hc was significant in W only. Moreover, HR was higher in Hx than Hc.

No significant difference was observed for RR and sigh frequency between the first and last hours of the recordings in Hc and Hx (P > 0.1). In contrast to Hc (P > 0.05), a decrease in apnea frequency between the first and last hours of the recordings was observed in W and QS (P < 0.03) during Hx. Finally, a decrease in HR was consistently observed between the first and last hours of the recordings in W and QS in Hx (P < 0.0001) and only during QS in Hc (P = 0.004).

NNS Activity

NNS frequency data are reported in Table 3 and Fig. 3. Overall, Hc increased the frequency of total and isolated NNS in W and QS compared with air. Although the same tendency was observed during AS, this increase was not statistically significant because of high variability between lambs. On the contrary, the overall effect of Hx was a tendency toward a decrease in the frequency of total and isolated NNS, which reached significance only for isolated NNS during QS. Finally, neither Hc nor Hx significantly modified NNS burst frequency. Overall, Hc and Hx had an inverse effect on NNS frequency, with amplitude of the effect being greater for Hc than for Hx. Total NNS frequency was identical in the first and last hours of the recordings in W and QS in Hc (78 ± 46 and 75 ± 43 h⁻¹ during W and 47 ± 40 and 39 ± 18 h⁻¹ during QS, P > 0.4) and Hx (44 ± 23 and 55 ± 29 h⁻¹ during W and 20 ± 16 and 26 ± 12 h⁻¹ during QS, P > 0.09).

NNS-breathing coordination data are reported in Table 4 and Fig. 4. In air, regardless of the state of alertness, i- and ie-type NNS frequencies were not significantly different. Both were decreased in Hc and increased in Hx, this was not related to a change in arousal frequency. Neither Hc nor Hx had a significant effect on %AS. Mean duration of W epochs was increased during Hc, but not Hx. Neither Hc nor Hx had a significant effect on the mean duration and frequency of QS and AS epochs.

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greater than e- and ei-type NNS frequencies. During Hx, alterations in NNS-breathing coordination, i.e., a reduction in ei-type NNS during AS and i-type NNS during QS, were inconsistent throughout all states of alertness. By contrast, irrespective of the state of alertness, Hc consistently increased ie-type NNS at the expense of i-type NNS, with the consequence that ie-type NNS was by far the most frequent. Interestingly, a strong positive correlation was observed between respiratory timing by NNS for the

**DISCUSSION**

The present study provides new insight into the differential effect of moderate Hc or Hx on NNS in the neonatal period. Hc increased overall NNS frequency as a result of a specific increase in ie-type NNS, regardless of the state of alertness. In contrast, Hx tended to decrease overall NNS frequency, with no effect on specific NNS types. Furthermore, neither Hc nor Hx altered the respiratory timing of the breath where NNS occurred or that of the preceding or subsequent breath. To our knowledge, this is the first study assessing the effect of Hx and Hc on spontaneous NNS, including NNS-breathing coordination. Uniqueness of the present results further stems from the fact they were obtained without sedation and throughout the three states of alertness.

**Sleep Architecture During Moderate Hypercapnia or Hypoxia**

Hypoxia and states of alertness. Results from the present study show an increase in the time spent in QS during Hx, with no changes in W or AS duration. These results are in general agreement with previous results in the perinatal period, showing longer epochs of QS in hypoxic kittens (2) and a decrease in the time spent in AS in hypoxic (anemia) fetal lambs (17). In addition, a recent study in adult humans showed that acute Hx increased total sleep time (12), which led the authors to suggest that accumulation of GABA released by hypothalamic ventrolateral preoptic neurons in Hx directly inhibits wake-promoting regions of the brain stem. Accordingly, Hx would promote and reinforce the sleep side of the hypothalamic sleep switch (39). Conversely, an increase in the time spent in W was reported in adult rats (21, 28, 37). Overall, discrepancies between studies may be related to the experimental design or differences in species and/or maturation, the latter being consistent with the concept of an enhanced hypoxic hypometabolism and inactivity/energy conservation as a protective response in the neonatal period (22).

### Table 4. Coordination between NNS and respiration in air, Hc, and Hx

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th></th>
<th>QS</th>
<th></th>
<th>AS</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD vs. Air vs. Hx</td>
<td>Mean ± SD vs. Air vs. Hx</td>
<td>Mean ± SD vs. Air vs. Hx</td>
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<td></td>
<td></td>
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<tr>
<td>Air</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>i-type</td>
<td>17±10</td>
<td></td>
<td>11±7</td>
<td></td>
<td>39±25</td>
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<tr>
<td>ie-type</td>
<td>22±12</td>
<td></td>
<td>11±8</td>
<td></td>
<td>33±19</td>
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</tr>
<tr>
<td>ei-type</td>
<td>6±5</td>
<td></td>
<td>4±5</td>
<td></td>
<td>9±6</td>
<td></td>
</tr>
<tr>
<td>e-type</td>
<td>3±24</td>
<td></td>
<td>2±1</td>
<td></td>
<td>6±3</td>
<td></td>
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<tr>
<td>Hc</td>
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<tr>
<td>i-type</td>
<td>14±12</td>
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<td>0.4</td>
<td></td>
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<tr>
<td>ie-type</td>
<td>44±20</td>
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<td>&lt;0.0001</td>
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<td>Hx</td>
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<tr>
<td>i-type</td>
<td>15±12</td>
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<td>6±6</td>
<td></td>
<td>37±26</td>
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<td>ie-type</td>
<td>20±10</td>
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<td>13±9</td>
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<td>37±26</td>
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</tr>
<tr>
<td>ei-type</td>
<td>3±3</td>
<td></td>
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<td></td>
<td>3±4</td>
<td></td>
</tr>
<tr>
<td>e-type</td>
<td>4±2</td>
<td></td>
<td>2±2</td>
<td></td>
<td>6±4</td>
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</tr>
</tbody>
</table>

n = 9. Respiratory phases are as follows: i-type, preceded by and followed by inspiration; e-type, preceded by and followed by expiration; ie-type, transition from inspiration to expiration; ei-type, transition from expiration to inspiration. *P < 0.05, i-type vs. e-type. **P < 0.05, i-type vs. ei-type. ***P < 0.05, i-type vs. ei-type. 

** Respiratory Timing and Ventilatory Resetting Before, During, and After NNS**

Respiratory timing data for the n − 1 and n + 1 breaths are reported in Fig. 6. Differences were considered physiologically significant upon reaching ≥20% higher than control values. Overall, regardless of NNS type, inspiratory gas condition, or state of alertness, swallowing did not alter respiratory timing for the n − 1 breath. Similar observations were made for the n + 1 breath, with the exception of ei-type NNS, which was often associated with moderate increases in Ti and Ttot. Conversely, respiratory timing of the breath where NNS occurred (n breath) was largely altered, depending on the NNS type. The following alterations were consistent, regardless of inspiratory gas condition and state of alertness, with a few exceptions in AS. Although i-type NNS was associated with an increase in Ti, Ttot, and Ti/Ttot, ie-type NNS was associated with an increase in Ti, Ttot, and Ti/Ttot, whereas e-type NNS was associated with an increase in Ti and Ttot and a decrease in Ti/Ttot. Finally, alterations of respiratory timing with ei-type NNS were highly variable. Neither Hc nor Hx modified alterations in respiratory timing by NNS for the n, n − 1, and n + 1 breaths.
Hypercapnia and states of alertness. Although earlier studies in adult rats suggested that Hc had no influence on sleep-wakefulness architecture (21, 37), our observation of an increase in the time spent in W is more consistent with the known stimulating effect of Hc on several chemosensitive neuronal populations known for stimulating W. These populations include noradrenergic neurons in the locus ceruleus and the paraventricular hypothalamic nucleus, serotonergic raphe neurons, histaminergic neurons in the tuberomammillary nucleus, and orexin/hypocretin in the hypothalamus (6, 9, 11, 24).

Cardiorespiratory Effects of Moderate Hypercapnia or Hypoxia

Respiratory effects of hypercapnia and hypoxia. Hypercapnia is a potent respiratory stimulus throughout life, including the fetal sheep (14) and the newborn lamb, from the very first hours after birth (31). The present results confirm that Hc increases RR in all three states of alertness, with a tendency to be less marked in AS, as previously reported in the newborn infant (7). Moreover, the increase in RR observed during Hx in the present study, regardless of the state of alertness, complements previous results in newborn lambs during W (5, 42). Hc and Hx have also been shown to increase RR in adult rats, regardless of sleep state (21). However, differences were reported in the magnitude of the increase in RR between QS and W in adult rats during Hx (28), whereas RR did not increase in adult dogs during Hx (30). Again, an explanation for these discrepancies may be related to species differences and/or differences in the experimental design.

Given that most apneas in the newborn lamb are postsigh apneas, the decreased frequency of apneas in W and AS during Hc, compared with air and Hx, appears to be mainly related to the simultaneous decrease in sigh frequency (Table 1). An Hc-related decrease in the sensitivity of the Hering-Breuer reflex, which is likely responsible for the postsigh apneas, is not supported by previous data in newborn rats (19).

Effect of hypercapnia and hypoxia on HR. In agreement with the present results, Hc and Hx have been previously reported to cause tachycardia during W in newborn lambs (16, 41). However, we are not aware of any previous data on the alteration of HR in Hx during sleep in newborn mammals. According to the present results, although HR was also increased in QS during Hc or Hx, relative to W, this increase in HR was abolished during AS. Unfortunately, the design of our study does not provide any clues as to the reasons for this AS effect.

Effects of Hypercapnia or Hypoxia on NNS Frequency

Increase in NNS frequency with hypercapnia. Results from the present study show that Hc significantly increased NNS frequency during W and sleep. This effect does not appear related to saliva production (13, 20), inasmuch as salivary flow did not appear to change in the present study (0.14 ± 0.06 and 0.14 ± 0.04 g/min in air and Hc, respectively). Importantly, CO2 has been shown to stimulate the activity of most laryngeal mechanoreceptors (1, 3, 4) and may stimulate activity of the swallowing CPG. Indeed, neurons of the swallowing CPG are colocalized with neurons of the respiratory CPG in the medulla, including those in the nucleus tractus solitarius and near the nucleus ambiguus (15). Moreover, previous studies suggest that swallowing and respiratory CPGs are incompletely separated in newborn animals (44, 45). Thus Hc might directly activate neurons of the swallowing CPG or indirectly activate neurons of the respiratory and swallowing CPGs via peripheral arterial chemoreceptors and/or various chemoreceptor sites in the brain stem.

The only two known prior studies on the effects of Hc on swallowing have yielded conflicting results. Indeed, Hc was reported to have no effect on the swallowing reflex triggered by electrical stimulation of the superior laryngeal nerve in anes-
Fig. 6. Influence of ii-, ie-, ee-, and ei-type NNS during Hx and Hc on respiratory timing parameters in the breath before ($n-1$), during ($n$), and after ($n+1$) the NNS. Ti, inspiratory time; Te, expiratory time; Ttot, total duration of the breathing cycle; Ti/Ttot, duty cycle. Open bars, air; solid bars, Hc; shaded bars, Hx. Values are expressed as percentage of the value obtained for the $n-3$ breath (i.e., control value) and averaged for the entire group. Open symbols indicate significant difference from control values; solid symbols indicate >20% difference from control values ($n=6$).
hypothesized, ventilated, and bivagotomized cats (26). On the contrary, Hc was later reported to decrease the frequency of water-induced swallowing in conscious adult humans (25). Although differences in swallowing responses to CO2 between adult humans or cats and newborn lambs may be related to differences in species and/or neural immaturity, important differences in experimental conditions are also likely involved.

Absence of effect of hypoxia on NNS frequency. In contrast to Hc, Hx did not significantly alter NNS frequency, regardless of the state of alertness, despite a significant increase in RR (although less marked than with Hc). Hx tended to decrease NNS frequency, which is in agreement with the decrease in swallowing frequency previously reported in anesthetized, bivagotomized cats under mechanical ventilation (26). The presence of anesthesia in the latter study may have enhanced the effect of Hx on swallowing activity. The reasons for the decrease in swallowing during Hx are unclear. 1) A decrease in saliva production in Hx is not supported by our measurements (0.14 ± 0.06 and 0.12 ± 0.02 g/min in air and Hx, respectively, in 3 lambs). 2) Although Hx could be hypothesized to inhibit the activity of the colocalized swallowing and respiratory CGPs, as reported for the hypoxic respiratory response in the newborn, this is also not supported by our results, which show a significant increase in RR in Hx compared with air. 3) The absence of an increase in NNS frequency in Hx, despite a significant increase in RR, may be related to the presence of hypocapnia in Hx (40 ± 1, 50 ± 1, and 30 ± 2 Torr in air, Hc, and Hx, respectively), which would specifically act on the swallowing reflex via an unknown mechanism. 4) The decrease in swallowing activity could be part of the more general decrease in respiratory-protecting reflexes, such as cough, previously reported in Hx (8, 46). Indeed, this may be linked to hypoxic hypometabolism, as measured in three lambs of the present study.

Coordination Between NNS and Respiration

The preponderance of inspiratory NNS vs. expiratory NNS observed in room air breathing in the present study, irrespective of the state of alertness, is in agreement with our previous studies in newborn lambs (34, 38). These results are at variance, however, with previous reports on NNS-breathing coordination showing a preponderance of e-type NNS with room air breathing (25). Such differences from our present results in lambs are not uniquely related to age, since NNS-respiration coordination in room air breathing in adult sheep and goats is identical to that observed in lambs (10, 36). Important differences in experimental conditions, i.e., study of water-induced swallowing in adult humans vs. spontaneously occurring NNS in the newborn and adult sheep and in goats, may be part of the explanation. In addition, species differences, including body position (sitting vs. “on all fours” or lying prone) are also potential causes.

The consistent, specific increase in ie-type NNS frequency in all three states of alertness during Hc is at variance with the only previous report in awake adult humans showing a complex alternation of NNS-breathing coordination in Hc, including an increase in i- and ei-type swallowing and a decrease in e-type swallowing (25). However, the specific increase in ie-type NNS in Hc in the present study is in partial agreement with previous results in adult humans showing that hyperventilation (without Hc) modifies NNS-breathing coordination by increasing e- and ie-type NNS (40). Additional support for the hypothesis that hyperventilation per se is linked to an increase in ie-type NNS is provided by the observation in the present study of a linear, highly significant relationship between RR and ie-type NNS frequency during air breathing. However, the absence of an overall increase in ie-type NNS percentage in Hx, despite significant hyperventilation, indicates that the effects of hyperventilation can be blunted by other influences such as hypocapnia.

Finally, the present observations that, overall, NNS modifies respiratory timing of the NNS breath only, with no ventilatory resetting in the following breath, especially for ie-type NNS in Hc, indicate the absence of ventilatory resetting in newborn lambs, regardless of the state of alertness or surrounding gas condition. These results are in agreement with previous identical data obtained in human infants during room air breathing (47). Previous data in adult mammals during room air breathing are controversial, showing a ventilatory resetting only after e-type NNS (a very rare NNS type in our study) in goats (10) or after all NNS types (29) or a total absence of ventilatory resetting, regardless of NNS type (27). These controversies are unexplained. To our knowledge, the present observations that Hc and Hx do not lead to ventilatory resetting after NNS are unique.

Clinical Relevance

It is unclear whether the increased NNS frequency during Hc has beneficial or deleterious effects during the neonatal period. For example, an increase in NNS frequency could reduce aspiration of pharyngeal reflexes. However, if they occur during AS, they can lead to obstructive apnea in preterm lambs (33). In addition, the long-term effect of increased NNS during prolonged Hc in the neonatal period on swallowing maturation is unknown.

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REFERENCES


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