Effects of postnatal smoke exposure on laryngeal chemoreflexes in newborn lambs

Marie St-Hilaire,1 Charles Duvareille,1 Olivier Avoine,1 Anne-Marie Carreau,1 Nathalie Samson,1 Philippe Micheau,2 Alexandre Doueik,3 and Jean-Paul Praud1

1Neonatal Respiratory Research Unit, Departments of Pediatrics and Physiology, 2Department of Engineering, and 3Department of Pathology, Université de Sherbrooke, Sherbrooke, Quebec, Canada

Submitted 10 December 2009; accepted in final form 16 September 2010

IN THE MATURE ORGANISM, stimulation of the laryngeal mucosa receptor by liquids triggers highly protective reflexes, termed laryngeal chemoreflexes (LCR), which consist of coughing, arousal, and swallowing to prevent tracheal aspiration. In the immature newborn mammal, however, LCR are rather associated with vagal components such as laryngospasm, apnea, bradycardia, and desaturation (51). The hypothesis that LCR can lead to apneas of prematurity, apparent life-threatening events, and sudden infant death syndrome (SIDS) stems from both clinical experience and experimental results (33, 40, 52). We previously showed that LCR are characterized by a major cardiorespiratory inhibition in preterm lambs (46). On the contrary, LCR in healthy full-term lambs are consistently similar to the mature LCR reported in adult mammals and characterized by lower airway protective mechanisms with minimal cardiorespiratory inhibition (45). These observations led us to hypothesize that the development of life-threatening cardiorespiratory events during LCR in a full-term newborn is promoted by certain neonatal conditions, such as postnatal smoke exposure.

After worldwide “Back to Sleep” campaigns, perinatal environmental tobacco smoke (ETS) exposure, especially during pregnancy, is now considered an important risk factor for SIDS (1, 2, 3, 8, 22, 23, 36, 44). Although SIDS has been reported to be increased two- to threefold by postnatal ETS in some studies (4), partitioning the effects of postnatal ETS may predispose to SIDS via enhancement of LCR (49). However, the clinical relevance of the high dose of nicotine used in that study was questioned by the authors themselves (49). In addition, studies on nicotine alone are likely insufficient to unravel all of the potential effects of ETS, given that cigarette smoke contains at least 4,000 different chemical compounds. Hence, the present study was aimed at testing our hypothesis that postnatal ETS, at a nicotine level equivalent to that reported in human infants, enhances cardiorespiratory inhibition during LCR. We also aimed at assessing whether the state of alertness or type of smoke used to trigger the LCR has an effect on the observed responses.

MATERIALS AND METHODS

Animals. Experiments were performed in 15 lambs born at term by spontaneous vaginal delivery and housed in our animal quarters (day-light cycle 0600–1800) during the days of the experimentation. Lambs were bottle-fed ad libitum with ewe milk three times a day at 8:00 AM, noon, and 5:00 PM. The protocol of the study was approved by the Ethics Committee for Animal Care and Experimentation of our institution.

Secondary tobacco smoke exposure. Eight lambs were exposed to secondary tobacco smoke (exposure group; 20 cigarettes/day) during the first 16 days of life. The 8-h daily exposure consisted of two periods of 4 h separated by a 30-min pause at noon to bottle-feed the lamb and collect a urine sample for cotinine measurements using a urine bag (U-Bag 24-Hour for newborn; Libertyville, IL). Before and after exposure, the lamb was bottle-fed, its body temperature and weight measured, and a blood sample collected for arterial gas measurements. A cigarette-smoking machine was built in collaboration with the Department of Mechanical Engineering of the Université de Sherbrooke. This custom-made equipment, which is fully programmable, smokes cigarettes automatically according to preset parameters and produces a mixture of mainstream and sidestream smoke (see Ref. 13 for further details). The latter was vented into a 1.2 × 1.2-m Plexiglas exposure chamber, where the nonrestrained lambs stayed for the duration of the exposure. Preliminary experiments had shown that...
smoking 20 cigarettes/day induced levels of urinary cotinine at the upper limits of the range reported in infants exposed to cigarette smoke postnatally at home (5, 6, 24, 29). Results obtained in the eight lambs exposed to secondary tobacco smoke were compared with a control group (7 lambs), which were exposed to air in the same exposure chamber for 8 h daily and during the first 16 days of life.

Instrumentation of the lambs and recording equipment. Surgery was performed on the 13th day of smoke exposure (2 days before the first recording) under general anesthesia (2% isoflurane, 30% N2O, and 68% O2), as previously described (46). Briefly, bipolar gold-plated stainless steel electrodes were inserted into the two thyroarytenoid muscles (Ta; a glottal adductor) for recording Ta electrical activity (Ta electromyogram; EMG). Three platinum needle electrodes were inserted, two into the parietal cortex for electroencephalogram (EEG) recording and one under the scalp as a ground. For electrocardiogram (ECG) recordings, two needle electrodes were inserted under the peristeum of the 5th ribs. Leads from all electrodes were subcutaneously tunneled to exit on the back of the lambs. In addition, a supraglottal catheter was inserted transcutaneously to allow injection of liquids onto the larynx 5–7.5 mm above the anterior part of the glottis. A plastic tubing was subcutaneously tunneled in the neck of the lamb and connected to the catheter (15). Finally, an arterial catheter was inserted into the carotid artery for recording systemic arterial pressure. Correct positioning of the electrodes and catheters was systematically verified at autopsy.

Lamb instrumentation was completed immediately before recording in nonsedated lambs. Nasal airflow was recorded by use of a thermocouple wire glued to the side of one nostril. Two platinum needle electrodes were placed subcutaneously near the right eye socket for electrooculogram (EOG) recording. Thoracic and abdominal elastic bands were placed for recording respiratory inductance plethysmography and to provide qualitative assessment of lung volume variations with respiration. A pulse oximeter probe (Masimo Radical; Irvine, CA) was attached at the base of the tail for continuous monitoring of oxygen hemoglobin saturation by pulse oximetry (SPO2) and pulse wave. Finally, the subcutaneous plastic tubing connected to the supraglottal catheter was attached to a custom-designed radiotelemetry-driven injector, where the liquid to be injected was warmed and maintained at the lamb’s body temperature. Leads from the EMG, EEG, EOG, and ECG electrodes and the nasal thermocouple were connected to a transmitter attached to the lamb’s back immediately before the experiment. Raw EMG, EOG, EEG, and ECG signal, as well as nasal flow, were transmitted by custom-made radiotelemetry equipment (34). The raw EMG signals were moving time averaged (100 ms). All variables were continuously recorded using an acquisition software (AcqKnowledge version 3.2; Biopac Systems, Santa Barbara, CA). Collected data were stored on compact disk for further analysis.

Design of the study. The study was designed to allow for simultaneous recording of Ta EMG, EOG, EEG, EOG, nasal flow, sum of thoracic and abdominal movements, and SPO2 while triggering LCR by injection of liquids onto the larynx. Recordings were performed in nonsedated lambs on postnatal days 15 and 16 in the evening following smoke exposure. The lambs were comfortably positioned on a mattress. Baseline values of heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP), SPO2, and temperature were first logged. Each recording day consisted of a random sequence of 0.5-ml injections via the supraglottal catheter of saline (0.9% NaCl, pH 5.5, osmolality = 326 mosM, [Cl–] = 181 mosM), distilled water (pH 5.7), HCl diluted in saline (pH 2, osmolality = 295 mosM, [Cl–] = 181 mosM), and ewe’s milk (pH 6.4 [SD 0.2], osmolality = 336 [SD 92] mosM, [Cl–] = 38 [SD 11] mosM). All solutions were injected once in both quiet sleep (QS) and active sleep (AS) on days 15 and 16 (total of 8 injections per each experimental day). The supraglottal catheter was systematically flushed with 1 ml of saline between two injections, and lambs were given at least 15 min of recovery time between two injections. Events such as cough, arousal, or full awakening were noted by an observer throughout the recording sessions.

Data analysis. The main objective of the study was to assess the effect of postnatal cigarette smoke exposure on LCR. In addition, we queried whether this effect was different in QS vs. AS and from one experimental solution to another. Analysis of the LCR was performed as described previously within the first minute following each laryngeal injection (45). First, the cardiorespiratory responses were assessed as follows. The percentage of decrease in HR [%dec HR = (HRBL – HR min)/HRBL × 100] was calculated, with HRBL representing the baseline HR value averaged over 1 min before injection and HR min representing the minimal HR value observed after injection. The percentage of increase in MAP, decrease in RR, and decrease in saturation were calculated in the same manner. Any presence of bradycardia (defined by a %dec HR >30%) was noted, and the number of bradycardias, total summed duration of bradycardias, and minimal HR value were tabulated. The number of apneas (defined as at least 2 missed breaths relative to baseline breathing) and the total summed duration of apneas were recorded. Moreover, the presence of any apneas longer than 5, 10, and 20 s was also noted. Respiratory LCR duration was measured as the time duration between the onset of the LCR and resumption of three consecutive breaths (53). The time spent with SPO2 below 90 and 85% was calculated, as well as the area under the same SPO2 values. In addition to cardiorespiratory responses, the number of swallows (recognized as a brisk high-amplitude and short-duration Ta EMG) (39) was tallied. In addition, total summed duration of Ta EMG (total Ta EMG duration, indicating laryngeal closure) was calculated. The presence of coughing was also inferred from visual observation and analysis of Ta EMG and respiratory inductance plethysmograph signals. Standard electro-physiological and behavioral criteria were used to define QS and AS from EEG, EOG, and continuous visual observation (41). Cortical arousal from QS was defined by the association of a change in EEG (decrease in amplitude + increase in frequency) for 3 s or more, with at least two of the following modifications: a 10% increase in HR, a change in RR, or a body movement (26). Arousal from AS was recognized by direct observation of the lamb and disappearance of intense EOG activity. Full awakening was defined when the lamb was still awake after 1 min (19). Finally, the number of stimulations with arousal or full awakening was documented.

Urinary cotinine was measured using an ELISA immunoassay kit (Bio-Quant COTININE Direct Elisa; San Diego, CA). Collected samples (3 ml) were stored at −20°C until analysis. Cotinine measurement was preferred to nicotine in this study because of its longer half-life (15–20 h vs. 30 min–2 h, respectively), slow renal elimination, and high urinary concentration (6–25 × nicotine concentration). Cotinine was assayed in the Department of Biochemistry of the Université de Sherbrooke Hospital using their standard protocol. Cotinine/creatinine ratio was then calculated.

The larynx was immediately removed after euthanasia and placed in a formaldehyde solution (1/10). Specimens were cut and placed in a cassette for dehydration and fixation in paraffin. Paraffin blocks were cut using a microtome, and slices were placed on a microscope slide for eosin-hematoxylin staining and subsequent observation. Laryngeal inflammation was assessed in three control lambs and four exposure lambs by a pathologist expert in larynx pathology, using a previously described inflammation score based on both epithelial and subepithelial changes (30).

Statistical analysis. Quantitative variables are expressed as means ± SD, whereas qualitative variables (coughing, arousal, and awakening) were expressed as relative frequency. Statistical analyses were performed on raw data for all variables. For baseline physiological values, the effect of exposure to smoke was assessed using the Student’s t-test. For the laryngeal chemoreflexes, quantitative variables were analyzed through a general linear model three-way ANOVA for repeated measures using Proc mixed procedures (SAS software version 9.1.3; Cary, NC) with group, solutions, and sleep
states as the independent variables. Qualitative variables were analyzed with a logistic regression model. Differences were deemed significant if \( P < 0.05 \). In addition, given the relatively small number of studied lambs (related to both the complexity of the ovine model and ethical constraints), it was decided to give full consideration to the presence of a significant trend, defined as \( P < 0.1 \).

RESULTS

A total of 95 stimulations were performed in the control group (QS: 14 saline, 14 distilled water, 13 HCl, 14 milk; AS: 12 saline, 10 distilled water, 9 HCl, 9 milk) and 116 stimulations in the exposure group (QS: 16 saline, 16 distilled water, 16 HCl, 16 milk; AS: 12 saline, 11 distilled water, 15 HCl, 14 milk). Because of technical reasons and difficulties in obtaining AS in some lambs, \%dec MAP and saturation indexes could only be measured in 2 and 4 lambs, respectively, of the control group in AS. All other measurements were successfully obtained in at least five lambs in both groups.

Passive smoking lamb model. All lambs completed the 16-day exposure to cigarette smoke without problems. No differences in arterial blood gases were apparent between the control and exposure groups (results not shown). However, an increase in spontaneous activity was observed in lambs from the exposure group, including head movements to verify sur- rounds and repeated jumps in the exposure chamber during 20- to 30-min periods. Mean weight increase was similar between both groups from the first postnatal day [control group: 3.1 (0.5) kg; exposure group: 3.6 (0.6) kg] to postnatal day 16 [control group: 5.6 (1.7) kg; exposure group: 6.4 (1.4) kg]. Baseline values of vital signs are detailed in Table 1: whereas RR and MAP were significantly higher in lambs from the exposure group, no differences in HR, \( \text{SpO}_2 \), and temperature were observed. The cotinine/creatinine ratio was significantly higher in the exposure group throughout the exposure \( [1,116 \text{ (832)} \text{ vs. 15 (12) ng/mg in the control group, } P < 0.0001] \), as well as during the last 2 recording days \( [891 \text{ (506)} \text{ vs. 11 (11) mg/mg in the control group, } P < 0.0001] \).

Effects of cigarette smoke exposure on LCR. Results obtained during LCR are given in Tables 2 and 3. After 2 wk of passive smoking, laryngeal stimulations (all solutions and both sleep states taken together) elicited significantly greater respiratory inhibition compared with the control group, including for the percent decrease in RR, apnea duration, and number of apneas. No interaction was found between groups, sleep states, and solutions for the first two variables. However, a significant interaction was found for the number of apneas in that the latter was greater in the exposure group only when LCR was triggered by water during active sleep (see Table 3). In addition, a tendency toward a greater cardiac inhibition in the exposure group was observed for the number of bradycardias, with no interaction between groups, sleep states, and solutions. No differences were observed for MAP, respiratory LCR duration, total duration of active glottal closure (measured by Ta muscle EMG), and \( \text{SpO}_2 \) variables. Some variables measuring protective mechanisms tended to be decreased in the exposure group, including the number of swallows and arousals. No differences were noted, however, for the number of coughs or awakening.

Apneas longer than 10 s were rare events in both the exposure (3 apneas) and control group (2 apneas), with none above 20 s. However, apnea duration between 5 and 9 s was more often observed in the exposure group than in the control group (48 vs. 21 apneas, respectively). In addition, all bradycardias but one were shorter than 5 s. One severe event was observed in one lamb in the exposure group following HCl injection in QS, with marked hypoventilation and a decrease in \( \text{SpO}_2 \) down to 76\% (Fig. 1).

Effects of passive smoking on laryngeal mucosa. There was no observable effect of cigarette smoke exposure on laryngeal inflammation in the few lambs studied. Indeed, histological analysis showed the presence of moderate inflammation in lambs from both the control (score 9/15, \( n = 3 \) lambs) and exposure groups (score 8/15, \( n = 4 \) lambs).

DISCUSSION

The present study provides novel findings on LCR triggered in nonsedated full-term lambs following exposure to passive smoking in the early postnatal period. Overall, our findings reveal that LCR after postnatal exposure to environmental

Table 2. Overall characteristics of LCR following 16-day exposure to cigarette smoke

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>%dec RR</td>
<td>51 (18)</td>
<td>67 (15)*</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>4.5 (8.3)</td>
<td>7.8 (8.5)*</td>
</tr>
<tr>
<td>No. of apneas</td>
<td>1.1 (2.7)</td>
<td>2.2 (4.7)*‡</td>
</tr>
<tr>
<td>%dec HR</td>
<td>21 (11)</td>
<td>28 (14)</td>
</tr>
<tr>
<td>Bradycardia duration, s</td>
<td>0.2 (0.7)</td>
<td>0.3 (0.7)</td>
</tr>
<tr>
<td>No. of bradycardias</td>
<td>0.2 (0.7)</td>
<td>0.5 (1)†</td>
</tr>
<tr>
<td>Minimum HR, min⁻¹</td>
<td>142 (9)</td>
<td>144 (7)</td>
</tr>
<tr>
<td>%inc MAP</td>
<td>23 (16)</td>
<td>18 (10)</td>
</tr>
<tr>
<td>Respiratory LCR duration, s</td>
<td>13.2 (10)</td>
<td>14.8 (8.8)</td>
</tr>
<tr>
<td>Total Ta EMG duration, s</td>
<td>2.2 (2.9)</td>
<td>2.5 (2.9)*</td>
</tr>
<tr>
<td>%dec Saturation</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Saturation, area under 90%</td>
<td>0.04 (0.2)</td>
<td>0.06 (0.3)</td>
</tr>
<tr>
<td>Saturation, area under 85%</td>
<td>0.01 (0.08)</td>
<td>0.02 (0.14)</td>
</tr>
<tr>
<td>No. of swallows</td>
<td>12 (10)</td>
<td>8 (7)†</td>
</tr>
<tr>
<td>Arousal no. of LCR with arousal/total no. of LCR</td>
<td>76/89</td>
<td>83/116†</td>
</tr>
<tr>
<td>Coughs (no. of LCR with cough/total no. of LCR)</td>
<td>14/94</td>
<td>11/116</td>
</tr>
<tr>
<td>Awakening (no. of LCR with awakening/total no. of LCR)</td>
<td>48/76</td>
<td>55/83</td>
</tr>
</tbody>
</table>

Values are means (SD). Results are means from all laryngeal stimulations (all solutions and all states of alertness) for each group. Values in the control group were available only in 5 lambs for percent increase in MAP (%inc MAP) and saturation indexes and in 6 lambs for the number of swallows and total duration of electrical activity of the thyroarytenoid muscle (Ta EMG duration). \%dec HR, \%dec RR, and \%dec saturation, percent decrease in HR, RR, or \text{SpO}_2 saturation, respectively; LCR, laryngeal chemoreflexes. *\( P < 0.05 \); †\( P < 0.1 \).
tobacco smoke are characterized by enhanced cardiorespiratory inhibition and decreased protective reflexes.

Passive smoking lamb model. Most studies on the effect of ETS exposure have focused on nicotine alone, despite the fact that tobacco smoke contains at least 4,000 different chemical compounds, many of which are known to be harmful to human health. In the present study, we used our custom-built smoking machine (13) to mimic postnatal parental smoking while ensuring that urinary cotinine level was relevant to previously published levels in infants (5, 6, 24, 29). The increase in baseline respiratory rate is in agreement with previous results on ETS exposure in both the human fetus and newborn (20). The absence of any increase in respiratory rate following the nicotine dose used in their study.

Effect of passive smoking on LCR. To our knowledge, despite its clinical importance, only one study has assessed the effect of perinatal ETS on LCR: passive smoking during gestation was shown to induce longer apneas during LCR in newborn rats only if combined with hyperthermia (53). Many methodological differences (prenatal exposure, sedation, injection of distilled water via tracheal catheter), in addition to species differences, can explain discrepant results with the present study. In keeping with our results, however, the authors observed no life-threatening events, even when ETS was added to hyperthermia.

The effect of nicotine alone on the cardiorespiratory components of the neonatal LCR has been addressed in three studies. In two studies conducted in sedated newborn piglets, the effect of one injection of nicotine on LCR was inconsistent, either showing no effect (16) or an enhanced cardiorespiratory inhibition (10, 11), this is the first observation of a decrease in swallowing induced by passive smoking. Relevance of this observation is related to the fact that decreased swallowing activity during LCR may lead to increased contact time between stimulating liquid and the laryngeal mucosa, leading in turn to enhanced LCR and deleterious cardiorespiratory

![Table 3](image)

### Table 3. Influence of sleep state and solution on the effects of 16-day exposure to cigarette smoke on LCR in lambs

<table>
<thead>
<tr>
<th></th>
<th>Quiet Sleep</th>
<th></th>
<th>Active Sleep</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>H₂O</td>
<td>HCl</td>
<td>Milk</td>
</tr>
<tr>
<td>Control</td>
<td>46.5 (21)</td>
<td>52 (14.5)</td>
<td>51.5 (19.5)</td>
<td>46.5 (20)</td>
</tr>
<tr>
<td>Exposure</td>
<td>56 (22.5)</td>
<td>69 (13.5)</td>
<td>67 (12)</td>
<td>70 (10.5)</td>
</tr>
<tr>
<td>%dec RR</td>
<td>0.9 (2)</td>
<td>0.8 (1)</td>
<td>1.5 (1)</td>
<td>0.8 (1)</td>
</tr>
<tr>
<td>No. of apneas</td>
<td>2.5 (7)</td>
<td>1 (0.5)</td>
<td>1.5 (1)</td>
<td>1.5 (0.9)</td>
</tr>
<tr>
<td>%dec HR</td>
<td>20.5 (7.5)</td>
<td>25 (13)</td>
<td>24 (6)</td>
<td>20 (9)</td>
</tr>
<tr>
<td>No. of bradycardias</td>
<td>16.5 (11)</td>
<td>21.5 (13.5)</td>
<td>28 (14.5)</td>
<td>20.5 (12)</td>
</tr>
<tr>
<td>%inc MAP</td>
<td>14 (9)</td>
<td>14.5 (11.5)</td>
<td>16 (18)</td>
<td>12.5 (11.5)</td>
</tr>
<tr>
<td>Respiratory LCR duration, s</td>
<td>10.5 (8.5)</td>
<td>13.5 (8.5)</td>
<td>12 (16)</td>
<td>10 (16)</td>
</tr>
<tr>
<td>No. of swallows</td>
<td>6 (4.5)</td>
<td>12 (10)</td>
<td>6 (3)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Arousal (no. of LCR with arousal/total no. of LCR)</td>
<td>9/12</td>
<td>12/13</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Coughs (no. of LCR with cough/total no. of LCR)</td>
<td>3/12</td>
<td>2/14</td>
<td>2/16</td>
<td>1/16</td>
</tr>
<tr>
<td>Awakening (no. of LCR with awakening/total no. of LCR)</td>
<td>7/10</td>
<td>11/12</td>
<td>6/9</td>
<td>4/7</td>
</tr>
<tr>
<td>%dec RR</td>
<td>18 (4.5)</td>
<td>21.5 (13.5)</td>
<td>22 (15)</td>
<td>17 (11)</td>
</tr>
<tr>
<td>%inc MAP</td>
<td>14 (9)</td>
<td>14.5 (11.5)</td>
<td>16 (18)</td>
<td>12.5 (11.5)</td>
</tr>
</tbody>
</table>

Values are means (SD). *Significant interaction between solution and sleep state.
events in the infant. Although we did not observe any differences in “coughing” in exposure lambs, we must recognize that our study did not allow us to fully assess coughing. Indeed, since we only counted the number of coughs and were not able to discriminate between “real” coughs and laryngeal expiratory reflexes (brisk expiration not preceded by a deep inspiration), we cannot dismiss a decrease in cough efficacy.

Our observation of decreased arousal during LCR following postnatal ETS may also be relevant with regard to the increase in the occurrence of SIDS after ETS. Indeed, arousal plays a critical role in homeostasis (22) and arousal deficiency is likely present in a number of SIDS victims (28, 37). Both maternal smoking and nicotine during pregnancy have been shown to affect arousal threshold (22, 42), but the present results represent the first account that postnatal passive smoking decreases arousal induced by laryngeal stimulation. Ability to arouse appears essential during LCR, by relieving cough suppression (32, 48), promoting swallowing (43), and cessation of apnea and bradycardia (50).

Our histological observations suggest that the effects of postnatal ETS on LCR are not related to laryngeal inflammation. In fact, the mechanisms involved in LCR alterations following ETS can be at play anywhere along the LCR neural circuitry, including from the laryngeal receptors to the central modulators of LCR. In addition, although a number of cigarette smoke constituents could contribute to these results, the majority of current knowledge comes from experiments on prenatal nicotine exposure. There is now abundant evidence that nicotine impairs brain development (14), including brain stem neuronal networks. For example, nicotine has been reported to alter brain stem nicotinic or serotoninergic receptors involved in the control of cardiorespiratory function, swallowing, and arousal (12, 14, 27), to increase GABA release and GABA_A receptor upregulation to respiratory centers (35, 54), and to decrease GABAergic input to cardioinhibitory vagal neurons (38). As for the other constituents of cigarette smoke, an interesting hypothetical mechanism for brainstem network dys-function relates to the induction of brain inflammation by NNK [4-methylnitrosamino-1-(3-pyridyl)-1-butanone] (18), which is reminiscent of the extensive brain stem inflammation reported in SIDS victims (25).

Influence of various solutions or sleep states on LCR. Aside from the number of apneas (increased in the exposure group with water and in AS only), no systematic effects of sleep states or solutions on the differences in cardiorespiratory events in the exposure vs. control group were observed. Of note, in a previous study, we also did not find any systemic effects of sleep states on LCR in preterm lambs (46). The fact that LCR can readily be induced by ewe’s milk, similar to water or HCl, is in agreement with previous reports in lambs, piglets, and puppies (7, 9, 21, 31, 47).

Clinical implications. Clinical evidence strongly suggests that LCR can be triggered by numerous liquids, including upper airway secretions, milk bottle-feeding, and both acidic and non-acidic laryngopharyngeal reflux (40). LCR are one of the mechanisms involved in apneas of prematurity, apparent life-threatening events, and probably some cases of SIDS (28, 33, 40). Many neonatal conditions (46, 51, 54) have been shown to enhance cardiorespiratory inhibition during LCR. The present findings show that postnatal ETS, at a level consistent with clinical observations, can also contribute in altering LCR toward more cardiorespiratory inhibition and less protective mechanisms. However, our results suggest that, at least in lambs, postnatal ETS is most often insufficient in itself to trigger potentially dangerous reflexes. Nevertheless, it is important to emphasize that most infants exposed to passive smoking have additional risk factors, which are likely synergistic with LCR for explaining the failing chain of events ultimately leading to SIDS. These include preterm birth, pathological laryngopharyngeal reflux with laryngitis, prone position, respiratory viral infection, and/or hyperthermia. Future studies need to consider these multiple factors. Finally, given that sheep are more

![Fig. 1. Cardiorespiratory reflexes triggered in a lamb at postnatal day 15 after 2 wk of exposure to cigarette smoke, following instillation of 0.5 ml of HCl onto laryngeal mucosa during quiet sleep. EEG, electroencephalogram; EOG, electrooculogram; Ta, electrical activity of the thyroarytenoid muscle; nasal flow, nasal airflow; lung volume, sum signal of the respiratory inductance plethysmograph, allowing qualitative measurement of respiration (inspiration upward); ECG, electrocardiogram; HR, heart rate (beats/min); AP, arterial pressure; SpO_2, oxygen hemoglobin saturation measured by pulse oximetry.](http://jap.physiology.org/content/109/6/1824/F1)
precocial than humans, repeating the experiments in our preterm ovine model (46) would also be worthwhile.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Jean-Philippe Gagné and Nathalie Samson, as well as Julie Hamon and Larissa Taker for statistical analyses.

GRANTS

The study was supported by grants from the Canadian Institutes for Health Research and the Foundation of Stars (Quebec) (to J.-P. Praud). A.-M. Carreau was an FRSQ-MSC Scholar of the Fonds de la Recherche en Santé du Québec (FRSQ). J.-P. Praud is a member of the FRSQ-funded Clinical Research Center Étienne-Le Bel, Sherbrooke University Hospital, and holder of the Canada Research Chair in Neonatal Respiratory Physiology.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


44. Slotkin TA. If nicotine is a developmental neurotoxicant in animal studies, dare we recommend nicotine replacement therapy in pregnant women and adolescents? *Neurotoxicol Teratol* 30: 1–19, 2008.


