RESEARCH ARTICLE

Reflex cardiorespiratory events from esophageal origin are heightened by preterm birth

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Nault S, Samson N, Nadeau C, Djeddi D, Praud J-P. Reflex cardiorespiratory events from esophageal origin are heightened by preterm birth. J Appl Physiol 123: 489–497, 2017. First published June 1, 2017; doi:10.1152/japplphysiol.00915.2016.—The involvement of gastroesophageal refluxes in cardiorespiratory events of preterm infants remains controversial. While a few studies in full-term newborn animals have shown that stimulation of esophageal receptors leads to cardiorespiratory reflexes, the latter remain largely unknown, especially after premature birth. The present study aimed to 1) characterize the cardiorespiratory reflexes originating from esophageal receptors in newborn lambs and 2) test the hypotheses that preterm birth enhances reflex cardiorespiratory inhibition and that C-fibers are involved in these reflexes. Eight full-term lambs and 10 lambs born 14 days prematurely were studied. Following surgical instrumentation, a 6-h polysomnography was performed without sedation to record electrocardiogram, respiratory movements, arterial pressure, laryngeal constrictor muscle activity, state of alertness, and hemoglobin oxygen saturation. Five esophageal stimulations of the upper and/or lower esophagus, including rapid balloon inflation and/or HCl injection, were performed in random order. A second recording was performed in full-term lambs 24 h later, after C-fiber blockade by capsaicin. Results confirmed that esophageal stimulations induced inhibitory cardiorespiratory reflexes combined with protective mechanisms, including laryngeal closure, swallowing, coughing, increased arterial pressure, and arousal. Preterm birth heightened cardiorespiratory inhibition. The strongest cardiorespiratory inhibition was observed following simultaneous stimulation of the lower and upper esophagus. Finally, cardiorespiratory inhibition was decreased after C-fiber blockade. In conclusion, esophageal stimulation induces inhibitory cardiorespiratory reflexes, which are partly mediated by C-fibers and more pronounced in preterm lambs. Clinical relevance of these findings requires further studies, especially in conditions associated with increased cardiorespiratory events, e.g., neonatal infection.

NEW & NOTEWORTHY Preterm birth heightens the cardiorespiratory events triggered by esophageal stimulation. The most extensive cardiorespiratory events are induced by simultaneous stimulation of the proximal and distal esophagus.

Gastroesophageal reflux; C-fibers

IMMATURITY OF THE OVERALL NERVOUS SYSTEM predisposes newborn infants to suffer from disorders such as cardiorespiratory events in preterm infants, apparent life-threatening events, and sudden infant death syndrome. Current knowledge suggests that gastroesophageal refluxes, which represent a common physiological event in healthy infants, are sometimes involved in these disorders via inhibitory cardiorespiratory reflexes and defective protective mechanisms. While the responsibility of laryngeal chemoreflexes, which can be triggered during laryngopharyngeal refluxes, is widely acknowledged (19, 52–54, 56), the causal relationships between reflexes originating from the esophagus during gastroesophageal refluxes and cardiorespiratory events remain highly controversial in the neonatal period (18, 34, 37, 38, 54).

Esophageal receptors and associated reflexes have been the focus of several physiological studies. Indeed, it has been documented in animals and full-term or preterm infants that the stimulation of chemo- and mechanoreceptors of the esophagus can trigger various reflexes (10, 12–17, 21, 23–26, 32, 51, 56). Moreover, the involvement of C-fibers, among others, in these reflexes has been suggested (25, 26). The above studies, however, have focused on reflexes modifying swallowing and/or esophageal function or those triggering bronchoconstriction and mucus production. Few physiological studies have addressed the possibility that esophageal stimulation can trigger cardiorespiratory events, especially in the neonatal period. Aside from beagle puppies where marked bradycardias were observed when a dilated balloon was slowly pulled through the esophagus or in response to upper esophageal distension (47), esophageal stimulations have been shown to induce mainly mild responses, namely cardiac decelerations or increased respiratory cycle duration, in sleeping preterm infants ready for home discharge (40, 41) or in anesthetized piglets (20).

The aims of the present study conducted in lambs were to: 1) characterize the cardiorespiratory reflexes originating from the esophagus upon mimicking of a gastroesophageal reflux in the neonatal period; this included testing the hypothesis that different stimulations and different regions of the esophagus can have an effect on the characteristics of the reflexes; 2) test the hypothesis that preterm birth is responsible for enhancing the reflex cardiorespiratory inhibition observed in response to esophageal stimulation; and 3) test the hypothesis that C-fibers are involved in these reflexes.

MATERIALS AND METHODS

The protocol of the study was approved by the Ethics Committee for Animal Care and Experimentation of the Université de Sherbrooke (protocol no. 283–15).
Animals

The experiments were conducted in 8 full-term lambs aged 4–5 days and weighing $3.6 \pm 1$ kg (2.3–4.6 kg) and 10 preterm lambs aged 8 days and weighing $4.0 \pm 1$ kg (2.8–5.4 kg). Preterm lambs were born 14 days prematurely as previously described (2). Briefly, premature labor was induced by mifepristone (8 mg/kg im) after stimulation of lung maturation by betamethasone (12 mg im $^2$). At birth, all preterm lambs were initially enrolled in a project on the effects of nasal continuous positive airway pressure on the introduction of bottle feeding (44). The last 10 preterm lambs of that initial study on bottle feeding were included in the present study.

Neonatal Ovine Model of Simulated Gastroesophageal Reflux

For the purpose of this study, a nonsedated ovine model of simulated gastroesophageal refluxes was designed to assess the inhibitory cardiorespiratory reflexes originating from esophageal receptors. A custom-made nasoesophageal catheter (length: 75 cm and diameter: 0.33 cm; Neurovent, Toronto, CA), comprised of two balloons (length: 2.5 cm and diameter with air: 1.3 cm) and two injections sites, was inserted in the esophagus of the animal. The distal balloon and injection sites were placed in the lower one-third of the esophagus, whereas the proximal balloon and injection sites were placed in the upper one-third of the esophagus. Correct placement of the esophageal catheter was validated by a chest X-ray before securing the catheter with an adhesive tape on the lamb’s muzzle (Fig. 1). To refute the hypothesis that the observed cardiorespiratory responses could originate from laryngeal chemoreceptors, verification that methylene blue injected via the upper port of the catheter did not reach the larynx was confirmed in three of the studied lambs. In addition, verification that no HCl penetrated in the larynx during upper acid instillation was also confirmed in three additional lambs using cineradiography [see Supplemental video 1 for an example of a cineradiography acquisition (Supplemental data for this article may be found on the journal website.)].

Chronic Instrumentation and Recording Equipment

Chronic surgical instrumentation was performed under general anesthesia (1–2% isoflurane, 30% O$_2$, and 68% medical air) in full-term lambs or under local anesthesia (5 mg/kg ketamine + 3 mg/kg im anafen and 2% lidocaine at incision sites) in preterm lambs. The procedure included insertion of 1) custom-built bipolar electrodes in both thyroarytenoid (a laryngeal constrictor) muscles for recording of electrical activity and 2) a catheter in the left carotid artery for monitoring systemic arterial blood pressure and for sampling arterial blood gases.

Instrumentation of the lamb was completed immediately before recordings and included 1) needle electrodes inserted subcutaneously for electroencephalogram (EEG), electrooculogram, and electrocardiogram recordings; 2) elastic bands installed on the chest and abdomen to monitor lung volume variations semi-quantitatively via respiratory inductance plethysmography; and 3) a pulse oximeter probe placed at the base of the tail for continuous monitoring of oxygen hemoglobin saturation (SpO$_2$). Physiological signals were transmitted wirelessly and continuously recorded (45). Finally, the

Fig. 1. X-ray images showing nasoesophageal catheter placement in a full-term lamb (x, superior and inferior injection sites; □, balloon location). The superior injection site and balloon were placed in the upper one-third of the esophagus, whereas the inferior injection site and balloon were placed in the lower one-third. The catheter is completely (top) or partially (bottom) filled with radio-opaque barium solution.
entire recording period was filmed with a webcam, and an experimenter was present throughout the recording to note all events.

Design of the Study

Full-term lambs. After a postoperative recovery period of 48 h, two polysomnographic recordings were performed in nonsedated animals on two consecutive mornings, before and after C-fiber (CF) blockade. Accordingly, CFs were blocked in the afternoon following the first polysomnographic recording by a subcutaneous injection of 25 mg/kg of capsaicin (diluted in 10% Tween 80, 10% ethanol, and 80% physiological saline) under 30 min general anesthesia. The integrity of bronchopulmonary CFs was assessed by inducing pulmonary chemoreflexes by intravenous injections of 5 and 10 μg/kg capsaicin under intact CF conditions, whereas effective CF blockade was verified by intravenous injections of 50 μg/kg capsaicin (5). Five esophageal stimulations were performed twice times (total of 10/lamb) during nonrapid eye movement (NREM) sleep in random order: 1) rapid balloon inflation (2 ml air × 10 s) in the upper esophagus; 2) rapid balloon inflation in the lower esophagus; 3) rapid injection of HCl (5 ml, pH 2) in the lower esophagus; 4) rapid injection of HCl (3 ml, pH 2) in the upper combined with balloon inflation in the lower esophagus (2 ml air); and 5) rapid injections of HCl (3 ml × 2, pH 2) in both the upper and the lower esophagus. Each animal was given at least 15 min of recovery time between two stimulations. Events such as agitation, cough, arousal, and/or full awakening were noted by an observer throughout the recording sessions. Finally, following completion of both recordings, euthanasia was performed by an intravenous injection of 90 mg/kg of pentobarbital sodium. Correct electrode and catheter positioning was systematically verified at necropsy.

Preterm lambs. After a postoperative recovery period of 24 h, esophageal stimulations were performed during one polysomnographic recording identically to that performed in full-term lambs. The lambs were euthanized immediately after this recording, without performing CF blockade.

Data Analysis

For each lamb, each of the five esophageal stimulations was repeated two times. Only the stimulation exhibiting the most marked inhibitory cardiorespiratory reflexes in each respective category was retained for subsequent analyses.

States of alertness. Standard electrophysiological and behavioral criteria were used to define NREM sleep. Cortical arousal from NREM sleep was defined by the association of a change in EEG (decrease in amplitude + increase in frequency) of 3 s or more with at least two of the following modifications: a 10% increase in heart rate (HR) or a change in respiratory rate or movement. Full awakening was defined when the lamb was still awake after 1 min (3).

Cardiorespiratory responses. Data collection was performed over 10 s immediately before each esophageal stimulation (baseline) and continued over the next 60-s period. The inhibitory cardiorespiratory responses to each esophageal stimulation that were calculated included (48): 1) the percent decrease in HR; 2) the number of cardiac decelerations (defined as a decrease in HR >30% for <5 s); 3) the number of bradycardias (defined as cardiac deceleration lasting at least 5 s); 4) the total duration of cardiac inhibition (including cardiac decelerations and bradycardias); 5) the minimum HR; 6) the number and total duration of apneas (defined as at least two missed breaths); 7) the percent decrease in O2 saturation, and 8) the minimum O2 saturation. The occurrence times of the minimum HR and the minimum O2 saturation were also analyzed.

Aside from the inhibitory cardiorespiratory responses, the percentage increase in mean arterial pressure (MAP), the maximum MAP, the number of swallows, the number of coughs, the total summed duration of laryngeal constrictor muscle activity, and the presence of arousal or full awakening were computed.

Statistical Analysis

Quantitative variables are expressed as medians (quartile 1, quartile 3), whereas binary qualitative data (arousal and full awakening, yes/no) are expressed as relative frequencies. Statistical analyses were performed on raw data for all variables. To assess the effect of the site of stimulation on inhibitory cardiorespiratory reflexes, a Friedman’s test followed by a post hoc analysis (Wilcoxon signed-rank test) was performed. To assess the effect of gestational age and involvement of CFs, a Wilcoxon signed-rank test was used. Finally, Fisher’s exact test and a McNemar test (differences between each esophageal stimulation) were used to analyze arousal or full awakening. All statistical analyses were performed with SPSS software (Armonk, NY), except McNemar test, which was performed with SAS software (Cary, NC). Differences were considered significant if P < 0.05.

RESULTS

Animals

The study was completed in 8 full-term lambs aged 4–5 days and weighing 3.6 ± 1 kg (2.3–4.6 kg) and 10 preterm lambs aged 8 days and weighing 4.0 ± 1 kg (2.8–5.4 kg).

Characterization of Reflexes from Esophageal Origin

Full-term lambs. As reported in Table 1, amplitude of the reflexes was variable from one lamb to the other. Overall, esophageal stimulations triggered inhibitory cardiorespiratory reflexes and protective mechanisms. For single esophageal stimulations, cardiac decelerations as well as coughing and laryngeal closure reflex were observed mostly after upper esophagus distension. Only one apnea or bradycardia was observed in two lambs after a single stimulation. Double stimulations triggered the most significant reflexes, with apneas and/or bradycardias in seven out of eight lambs, as well as a greater increase in mean arterial pressure and heightened swallowing activity compared with single stimulation.

Arousal was nearly always present during both upper and lower esophagus distension (single stimulations), but not during HCl injection. Full awakening occurred almost solely during double stimulations.

Preterm lambs. Overall, as shown in Figs. 2 and 3, prematurity birth heightened the reflexes triggered by esophageal stimulations. As presented in Table 2, all types of esophageal stimulations induced apneas in some preterm lambs, with their duration being more substantial with double stimulations. Heart decelerations were observed with all types of stimulations in some preterm lambs (not always in the same lambs as observed for apnea, however) and were overall significantly greater than in full-term lambs. In preterm lambs, single upper esophageal distension induced cardiac decelerations as well as apneas, coughing, and laryngeal closure reflex (see Fig. 4). Bradycardias were present mainly for double stimulations and in 9/10 preterm compared with 5/8 full-term lambs. A decrease in SpO2 was present in some lambs with all stimulations, culminating after double stimulations. An increase in mean arterial pressure was present in all preterm lambs with all stimulations.

Coughing and mostly swallowing activity were observed with double-esophageal stimulations. As in full-term lambs, arousal was very frequent in response to single stimulation, except for HCl injection, whereas awakening was most often present with double stimulations.
### Table 1. Reflexes from esophageal origin and effect of C-fiber blockade in full-term lambs

<table>
<thead>
<tr>
<th></th>
<th>Upper Distension After CFs blockade</th>
<th>Lower Distension After CFs blockade</th>
<th>Lower HCl Injection Before CFs blockade</th>
<th>Lower HCl Injection After CFs blockade</th>
<th>Lower Distension and Upper HCl Injection Before CFs blockade</th>
<th>Lower Distension and Upper HCl Injection After CFs blockade</th>
<th>Upper and Lower HCl Injection Before CFs blockade</th>
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Results are presented as medians (quarter 1, quarter 3); \( n = 8 \) full-term lambs for each group. CF: C-fibers; HR, heart rate; SpO\(_2\), hemoglobin saturation; Min, minimal; Max, maximum; MAP, mean arterial pressure; EAta, electrical activity of the thyroarytenoid muscle. \( P < 0.05 \), t-s. lower HCl injection, t-s. lower distension + upper HCl injection, t-s. upper + lower HCl injections. **P < 0.05 vs. before C-fiber blockade.

### Involvement of C-Fibers on Esophageal Reflexes in Full-Term Lambs

Overall, because of limited responses with single stimulations, significant differences were mostly observed with double stimulations. As reported in Table 1, a significant decrease \( (P \leq 0.03) \) in post- vs. predesensitization was observed for the number of apneas and total apnea duration, as well as for the amplitude of cardiac decelerations for double HCl stimulations.

The effect of C-fiber blockade on the increase in mean arterial pressure, laryngeal closure duration, swallowing activity, and coughing was variable from one stimulation to another. C-fiber blockade significantly enhanced the percentage increase in mean arterial pressure after upper esophageal distension \( (P = 0.03) \). No statistical difference was observed for the arousal and full awakening responses.

### DISCUSSION

Overall, our findings reveal that for the first time that the cardiorespiratory inhibition triggered by esophageal stimulation is heightened in preterm compared with full-term lambs. Furthermore, simultaneous stimulation of the proximal and distal esophagus, as observed during proximal gastroesophageal reflux, leads to greater cardiorespiratory inhibition than a single stimulation limited to either the proximal or distal esophagus. Finally, our results suggest that C-fibers are partly responsible for these cardiorespiratory reflexes.

### Literature Data on Reflexes Originating from the Esophagus

Previous physiological studies have clearly shown that various reflexes can originate from the stimulation of esophageal mechanoreceptors and/or chemoreceptors, as summarized recently (46). Most studies, however, have primarily focused on understanding the alterations of esophageal function in response to gastroesophageal reflexes.

**Reflexes triggered by mechanical stimulation.** Studies in anesthetized or decerebrate adult cats have shown that slow esophageal distension leads to reflexes such as upper esophageal sphincter contraction, secondary peristalsis and lower esophageal sphincter relaxation, as well as to a pharyngeal swallow response. Conversely, it has been shown that rapid esophageal distension induces reflex relaxation of the upper esophageal sphincter and belching (23, 24, 26, 27), as well as active laryngeal closure (esophagoglottal closure reflex) (24, 26, 51).

Studies in humans have reported similar reflexes in response to slow esophageal distension in full-term and preterm infants (8, 11, 12, 15, 17) in addition to esophagoglottal closure in response to rapid esophageal distension in adults and full-term (50) and preterm infants (13, 14).

**Reflexes triggered by chemical stimulation.** Studies in decerebrate adult cats have revealed that infusion of water or acid induces secondary peristalsis and the pharyngeal-swallow response (22, 27). Moreover, injection of acid in the esophagus has been shown to induce swallowing in full-term piglets (32).

Studies in human preterm newborns have shown that water or apple juice infusion induces secondary peristalsis, swallowing, upper esophageal sphincter contraction, and lower esophageal sphincter relaxation (11, 15, 17), as well as arousal (12).

**Cardiorespiratory responses.** Cardiorespiratory responses to esophageal stimulation have been mainly assessed in the neonatal period, albeit in only a few studies.
Studies in nonsedated, sedated, or anesthetized newborn piglets born at term have reported apnea in response to acid infusion in the upper or the lower esophagus (19, 32), as well as cardiac decelerations (20). One study in beagle puppies aged 4–5 wk has shown that acute esophageal dilatation can induce cardiac responses, which included cardiac arrhythmias, either supraventricular or ventricular, up to ventricular fibrillation and at times cardiovascular collapse (47). In preterm infants during rapid eye movement sleep, distension or acid infusion in the lower esophagus has been observed to prolong both respiratory cycle duration and RR interval (40, 41).

Conversely to the cardiorespiratory inhibitory responses reported in the neonatal period, noxious esophageal stimuli have been shown to induce an increase in arterial blood pressure and heart rate in adult anesthetized or decerebrate rats, via both a sympathetic and a vagal pathway (30, 36).

New Knowledge Revealed by the Present Study

Results of the present study on cardiorespiratory reflexes from esophageal origin are reminiscent of our previous observations on laryngeal chemoreflexes in preterm vs. full-term lambs (52, 53). Immaturity of the brain stem cardiorespiratory “controllers” (6), perhaps with increased esophageal receptor sensitivity and/or immature control by higher brain centers (48), is likely involved in the heightened inhibitory responses observed in preterm lambs. Moreover, the observation that double stimulations induced the most powerful inhibition may be related to the summative effect of simultaneous stimulation of the distal and proximal esophagus. While not contradictory, our results, however, do not yield evidence to support results in adult humans suggesting heightened sensation of the proximal compared with the distal esophagus (57, 58).

Our results also demonstrate that esophageal stimulation elicits airway-protective reflexes such as 1) swallowing and arousal; 2) glottal closure, similar to that triggered by rapid esophageal distension in adults and infants (13, 14, 50); and 3) coughing. Interestingly, these airway-protective mechanisms were observed in some lambs following upper esophageal distension only (not HCl injection), proving that they can originate from the esophagus. This is probably related to the common embryonic origins of the digestive and respiratory tract with a shared vagal innervation (9, 31).

Fig. 2. Sample tracings showing cardiorespiratory inhibitory reflexes during simultaneous lower and upper HCl injection in one full-term (A) and one preterm (B) lamb. Heightened and clinically significant cardiorespiratory responses are observed in the preterm lamb. HR, heart rate; BPM, beats/min; EEG, electroencephalogram; EOG, electrooculogram; ECG, electrocardiogram; EAta, electrical activity of the thyroarytenoid muscle; +EAta, moving time averaged EAta; SpO2, oxygen hemoglobin saturation; respiratory movements, sum signal of the respiratory inductance plethysmography; ⊧, cough; ⊕, body movements.
Finally, esophageal stimulation induced an increase in mean arterial pressure in all lambs because of sympathetic activation. In fact, similarly to the laryngeal chemoreflexes (1, 52, 53), esophageal stimulation is responsible for a sympatheticovagal coactivation, which undergoes a developmental maturation. Indeed, while the vagal component (apnea, bradycardia) predominates in preterm compared with full-term lambs, the sympathetic component is prominent in adult mammals (30, 36).

Involvement of C-Fibers

Our present results suggest that C-fibers are partly involved in the cardiorespiratory reflexes originating from the esophagus, as suggested previously for other esophageal reflexes (24, 26). It is known that the transient receptor potential cation channel subfamily V member 1 (TRPV1), present on C-fibers, is activated by a large array of noxious agents, such as extracellular H⁺ and rapid air distension (4, 28, 42). Our results show that the cardiorespiratory inhibition induced by double-esophageal stimulation was blunted by selective blockade of C-fibers. The overall message is, however, less clear for the other components of the reflexes, especially mean arterial pressure and cough. We believe this reflects the fact that only TRPV1 were blocked, leaving numerous other esophageal receptors still functional (see Table 1 in Ref. 33 for entire list).

Potential Clinical Relevance of the Present Physiological Results

There is general agreement that laryngopharyngeal reflexes can be responsible for some cases of cardiorespiratory events in preterm infants as well as apparent life-threatening events and probably sudden infant death syndrome (reviewed in Refs. 29, 38, and 55). Conversely, studies in preterm infants have led to diverse interpretations with regard to the causal relationships between gastroesophageal reflexes and events (18, 37, 38, 49). Available studies in this latter population are scarce and have used sedation/anesthesia and/or did not include sufficiently immature newborns (19, 20, 32, 47). Although any translation to human infants must be considered with great caution, our unique results support the view that gastroesoph-
Ageal reflux could be responsible for cardiorespiratory events in preterm infants or infants with apparent life-threatening events (7, 48). Conditions featuring abnormal esophageal motility, e.g., resulting from prematurity (35) or esophagitis (48), could favor such occurrence by increasing the contact time of a refluxed bolus with esophageal receptors.

Limitations of the Study

The present study has several limitations. First, esophageal stimulations were performed only during sleep. Given that cardiorespiratory events in preterm infants are more often related to gastroesophageal reflux during wakefulness (39), further studies will need to include awake preterm lambs. Second, given that the majority of refluxes in newborns are weakly acid (43), further studies in lambs will have to assess the effects of a weakly acid-liquid stimulus. Finally, an important limitation of our study is the lack of documentation in all studied lambs that the HCl injected in the upper esophagus did not penetrate in the larynx, inducing in turn laryngeal chemoreflexes (vs. esophageal chemoreflexes). This nonpenetration was nevertheless confirmed in three lambs using methylene blue and in three additional lambs via cineradiography, thus

Table 2. Reflexes from esophageal origin in preterm lambs

<table>
<thead>
<tr>
<th>Reflexes from esophageal origin in preterm lambs</th>
<th>Upper Distension</th>
<th>Lower Distension</th>
<th>Lower HCl Injection</th>
<th>Lower Distension and Upper HCl Injection</th>
<th>Upper and Lower HCl Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of apneas</td>
<td>1 (0;1)</td>
<td>1 (0;1)</td>
<td>1 (0;1)</td>
<td>2 (1;2)</td>
<td>2 (1;2)</td>
</tr>
<tr>
<td>Total apnea duration, s</td>
<td>2.2 (0;6.5)</td>
<td>1.5 (0;4)</td>
<td>1.5 (0;4)</td>
<td>6.5 (4;12.1)</td>
<td>7.5 (5.4;10.9)</td>
</tr>
<tr>
<td>HR decrease, %</td>
<td>46 (42;61)</td>
<td>48 (42;52)</td>
<td>37 (26;40)†§</td>
<td>63 (52;67)</td>
<td>64 (54;72)</td>
</tr>
<tr>
<td>No. of bradycardias</td>
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<td>0 (0;0)</td>
<td>0 (0;0)</td>
<td>1 (0;2)</td>
<td>1 (0;2)</td>
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<tr>
<td>Total bradycardia durations, s</td>
<td>0 (0;0)</td>
<td>0 (0;0)</td>
<td>0 (0;0)</td>
<td>7.9 (0;20.3)</td>
<td>9 (0;15.7)</td>
</tr>
<tr>
<td>Occurrence time of min HR, s</td>
<td>7 (5;9)†</td>
<td>5 (1;15)‡</td>
<td>21 (10;40)</td>
<td>8 (2;29)</td>
<td>16 (9;23)</td>
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<tr>
<td>No. of cardiac decelerations</td>
<td>3 (1;4)</td>
<td>4 (1;7)</td>
<td>1 (0;1)†</td>
<td>8 (4;13)</td>
<td>5 (1;8)</td>
</tr>
<tr>
<td>Total cardiac inhibition duration, s</td>
<td>3.2 (1.9;6.2)</td>
<td>3.3 (1.4;7)</td>
<td>0.2 (0.0;8)†§</td>
<td>28.2 (4.4;32.5)</td>
<td>10.6 (4;33)</td>
</tr>
<tr>
<td>SpO2 decrease, %</td>
<td>3 (1;4)</td>
<td>4 (2;7)</td>
<td>3 (1;6)</td>
<td>11 (2;15)</td>
<td>6 (4;12)</td>
</tr>
<tr>
<td>Min SpO2, %</td>
<td>87 (87;92)</td>
<td>87 (84;94)</td>
<td>87 (84;93)</td>
<td>82 (76;89)</td>
<td>85 (76;89)</td>
</tr>
<tr>
<td>Occurrence time of min SpO2, s</td>
<td>28 (23;40)</td>
<td>49 (17;53)</td>
<td>53 (16;55)</td>
<td>43 (36;44)</td>
<td>43 (24;57)</td>
</tr>
<tr>
<td>No. of desaturations &gt;3%</td>
<td>0 (0;1)</td>
<td>1 (0;1)</td>
<td>0 (0;1)</td>
<td>1 (0;1)</td>
<td>1 (1;1)</td>
</tr>
<tr>
<td>No. of desaturations &gt;4%</td>
<td>0 (0;0)</td>
<td>1 (0;1)</td>
<td>0 (0;1)</td>
<td>1 (0;1)</td>
<td>1 (0;1)</td>
</tr>
<tr>
<td>MAP increase, %</td>
<td>22 (20;24)†</td>
<td>21 (13;27)‡</td>
<td>17 (14;20)†</td>
<td>42 (36;59)</td>
<td>53 (35;64)</td>
</tr>
<tr>
<td>Max MAP, mmHg</td>
<td>85 (74;95)†</td>
<td>86 (75;93)‡</td>
<td>80 (71;85)†</td>
<td>99 (87;116)</td>
<td>113 (89;124)</td>
</tr>
<tr>
<td>Occurrence time of max MAP, s</td>
<td>7 (3;13)</td>
<td>9 (7;15)</td>
<td>7 (6;33)</td>
<td>10 (6;20)</td>
<td>12 (8;16)</td>
</tr>
<tr>
<td>Total EAta duration, s</td>
<td>3 (1;4)</td>
<td>3 (1;8)</td>
<td>2 (1;3)§</td>
<td>6 (3;18)</td>
<td>8 (4;16)</td>
</tr>
<tr>
<td>No. of swallows</td>
<td>0 (0;1)</td>
<td>1 (0;4)</td>
<td>1 (0;3)</td>
<td>6 (1;6)</td>
<td>3 (1;5)</td>
</tr>
<tr>
<td>No. of coughs</td>
<td>0 (0;1)</td>
<td>0 (0;0)§</td>
<td>0 (0;0)§</td>
<td>4 (2;7)</td>
<td>4 (2;7)</td>
</tr>
<tr>
<td>Arousal</td>
<td>9/10</td>
<td>8/10</td>
<td>0/10</td>
<td>5/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Full awakening</td>
<td>1/10</td>
<td>1/10</td>
<td>3/10</td>
<td>5/10</td>
<td>7/10</td>
</tr>
</tbody>
</table>

Results are presented as medians (quartile 1, quartile 3); n = 10 preterm lambs for each group. *P < 0.05, ‡vs. lower HCl injection, †vs. lower distension + upper HCl injection, and §vs. upper + lower HCl injections.

Fig. 4. A single upper esophageal distension induces cardiac decelerations as well as apneas and laryngeal closure reflex in a premature lamb. See Fig. 2 for abbreviations.
leaving this possibility unlikely. In addition, the observation that a distention strictly limited to the upper esophagus triggered aperistalsis constitutes clear evidence that a stimulation restricted to the esophagus can induce clinically significant cardiorespiratory reflexes.

Conclusions

Esophageal stimulation triggers heightened cardiorespiratory events in preterm compared with full-term lambs, probably because of the overall immaturity of the nervous system. The most clinically significant events are induced by simultaneous stimulation of the proximal and distal esophagus. Although any translation to human newborns must be made with great caution, we propose that the present physiological results obtained in lambs justify further testing the hypothesis that gastroesophageal reflexes can trigger cardiorespiratory events in certain preterm infants and under certain conditions. Forthcoming studies will, however, need to first ascertain that each and every stimulation is restricted to the esophagus while excluding any laryngeal penetration.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.N., N.S., and C.N. performed experiments; S.N. and C.N. analyzed data; S.N., N.S., D.D., and J.-P.P. interpreted results of experiments; S.N. and C.N. prepared figures; S.N. and N.S. drafted manuscript; S.N., N.S., D.D., and J.-P.P. edited and revised manuscript; S.N., N.S., D.D., and J.-P.P. approved final version of manuscript.

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