Université de Sherbrooke

Chémoréflexes laryngés induits par l'acide, l'eau *vs* le salin chez les agneaux nouveau-nés durant le sommeil calme

par

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Liste des abréviations

CRL : Chémoréflexes laryngés

VAI : Voies aériennes inférieures

ALTE : Malaises graves du nourrisson

MSN : Mort subite du nourrisson

Ta : Muscle thyroaryténoïdien

PA : Pression artérielle

Fr : Fréquence respiratoire

Fc : Fréquence cardiaque

ASIC : Acid sensing ion channels

VR : Récepteurs vanilloïdes

Résumé

Mise en contexte : Les chémoréflexes laryngés (CRL) sont déclenchés suite au contact entre un liquide et la muqueuse laryngée. Chez un organisme mature, ces CRL sont responsables de mécanismes de protection des voies aériennes inférieures (VAI) tels que déglutitions, toux et réaction d'éveil afin d'éviter l'aspiration. Par contre, chez un organisme immature comme c'est le cas chez les nouveau-nés, ces CRL associent apnée, bradycardie, laryngospasme, hypertension et redistribution du débit sanguin. En période néonatale, ces CRL, déclenchés en réponse à un reflux gastro-œsophagien acide, sont tenus responsables d'apnées du prématuré, de malaises graves du nourrisson (ALTE) et probablement de quelques cas de mort subite du nourrisson (MSN). Malgré leur pertinence clinique évidente, la revue de la littérature permet de constater que de nombreuses questions persistent concernant les CRL, principalement parce que les conditions expérimentales des études antérieures ne reflètent pas ce qui est vu en clinique. Ainsi, les CRL ont été étudiés le plus souvent en utilisant des modèles anesthésiés, en utilisant l'eau distillée, en se servant d'une trachéotomie pour l'injection des solutions et finalement en ne prenant pas en compte les stades de conscience. Une meilleure compréhension des CRL, en particulier déclenchés par des solutions acides, est donc nécessaire.

But du projet : Le but de ce travail est d'étudier les CRL chez l'agneau nouveau-né sans sédation en réponse à l'acide, en comparaison à l'eau distillée et au salin durant le sommeil calme.

Méthodologie : Au deuxième jour de vie post-natale, une chirurgie a été effectuée. Elle a permis l'installation d'électrodes afin d'enregistrer l'activité de certains muscles (muscle constricteur du larynx : muscle thyroaryténoïdien, Ta, et diaphragme) et des stades de conscience. Un cathéter artériel (enregistrement de la pression artérielle, PA) et un cathéter supra-glottique (injection des liquides) ont été installés. Des instrumentations non-invasives ont également été ajoutées les journées d'expérimentation : thermocouple nasal (fréquence respiratoire, Fr), pléthysmographie par inductance (Fr), électrocardiogramme (fréquence cardiaque, Fc) et finalement oximétrie pulsée (saturation). Au quatrième et cinquième jour de vie post-natale, il y a eu induction des CRL. Les CRL ont été déclenchés par 0.5 ml d'eau distillée, de solutions acides (HCl et acide citrique dilués dans l'eau et le salin) et de salin *via* le cathéter supra-glottique. Chaque journée d'expérimentation comprenait l'injection de chaque solution (en double) et ce de façon randomisée durant le sommeil calme. Suite aux différentes stimulations laryngées, les réponses cardiorespiratoires et les mécanismes de protection des VAI obtenus ont été analysés (ANOVA et SAS) et comparés au contrôle (salin).

Résultats : Chez l'agneau à terme sans sédation, les réponses cardiorespiratoires (diminution de la Fr et de la Fc, augmentation de la PA) suite aux différentes stimulations sont légères et grossièrement similaires, que la stimulation soit de l'eau distillée ou des solutions acides. Toutefois, déglutitions, toux, réaction d'éveil et réveil complet sont plus marqués suite à l'injection des solutions acides comparativement au salin et à l'eau distillée.

Conclusion : Cette étude apporte des résultats uniques sur les CRL chez l'agneau à terme en santé et permet de conclure que les réflexes déclenchés chez les agneaux nouveau-nés à terme en santé sont de type mature. Les études futures permettront d'étudier chez les agneaux à terme et prématurés l'influence de certains facteurs externes, tels le tabagisme passif, l'infection des voies aériennes supérieures ou l'inflammation laryngée secondaire au reflux gastro-oesophagien, sur les CRL.

Introduction

La muqueuse laryngée comprend une densité très importante de récepteurs, incluant plusieurs types de mécanorécepteurs et de chémorécepteurs. Continuellement, les afférences provenant des mécanorécepteurs – récepteurs à pression, à proprioception ou à température – modifient la commande respiratoire centrale et modulent finement le rythme, la fréquence et l'amplitude ventilatoire. Les afférences provenant des chémorécepteurs, quant à elles, sont responsables de réflexes de protection de voies aériennes inférieures (VAI), et ne sont pas impliquées dans la modulation fine de la respiration. La stimulation de ces chémorécepteurs, provoquée par exemple par des sécrétions locales ou par l'ingestion de liquide, induit des déglutitions, et, si nécessaire, de la toux, afin d'éviter l'aspiration dans les VAI. Chez un sujet endormi, la stimulation de ces récepteurs provoque en plus des réflexes énumérés précédemment des réactions d'éveil et/ou agitation afin, encore une fois, d'éviter l'aspiration. Chez les mammifères, ces réflexes nommés les chémoréflexes laryngés (CRL), ne sont pleinement efficaces qu'après une maturation du système nerveux (Thach, 2001). En période néonatale, en raison d'une immaturité rhombencéphalique, des chémoréflexes laryngés immatures et plus complexes sont fréquemment observés. Ces réflexes d'origine vagale sont bien souvent délétères et associent laryngospasme, apnée, bradycardie. De surcroît, des composantes sympathiques, c'est-à-dire des réflexes un peu plus positifs, viennent s'ajouter aux réflexes délétères et associent hypertension et redistribution du débit sanguin.

Les CRL, chez les nouveau-nés, sont la conséquence probable de mécanismes de protection pulmonaire retrouvés chez le fœtus et qui persistent au-delà de la naissance (Thach, 2001). Alors qu'il est encore en plein développement, le fœtus est entouré de liquide amniotique qu'il peut, dès la vingtième semaine de gestation, déglutir régulièrement permettant ainsi le maintien d'un volume intra-utérin approprié (Chan et al, 1997 ; Ross and Nijland, 1998). Ces phénomènes apparaissent presqu'en même temps que le fœtus exerce ses premiers mouvements respiratoires (Thach, 2001). Le fœtus doit donc se prémunir afin d'éviter l'aspiration du liquide amniotique dans les VAI, évènement qui pourrait être délétère. Le fœtus s'est ainsi muni de système efficace afin d'éviter l'aspiration de toute substance dont la composition diffère du liquide présent dans les VAI autrement dit de toute substance qui diffère du liquide pulmonaire. Par conséquent, des apnées prolongées sont fréquemment observées en période prénatale, mais elles ne représentent aucun risque pour le fœtus dont les échanges gazeux s'effectuent encore *via* le lien maternel. Ce système dont s'est doté le fœtus pour éviter l'aspiration demeure par contre toujours présent à la naissance, mais constitue maintenant un paradoxe. En effet, le but d'éviter l'aspiration est toujours le même, mais provoque maintenant des conséquences plus dramatiques voire même mortelles chez les nourrissons dont les échanges gazeux s'effectuent maintenant *via* les voies aériennes. Ces réflexes cardiorespiratoires délétères semblent, par contre, s'atténuer avec l'âge post-natal, laissant ainsi la place à des mécanismes de protection des VAI (déglutitions, toux, réaction d'éveil) plus adaptés à la situation.

Les néonatalogistes et les pédiatres connaissent trop bien les conséquences que peuvent avoir les CRL sur la vie des nourrissons. En clinique, les CRL peuvent être déclenchés, en autre, par un reflux gastro-œsophagien acide et sont impliqués dans les apnées du nouveau-né prématuré, les malaises graves du nourrisson (ALTE), et font partie des hypothèses pathogéniques du syndrome de mort subite du nourrisson (MSN) (Wetmore, 1993 ; Page and Jeffery, 2000 ; Thach, 2001).

Certains facteurs extérieurs semblent aggraver la fréquence et la sévérité des conséquences cardiorespiratoires des CRL et ainsi conduire aux conséquences dramatiques rappelées ci-dessus. Ces facteurs comprennent par exemple, l'emploi d'anesthésique ou de médicaments dépresseurs respiratoires, la prématurité (Marchal et al 1982 ; Thach, 2001), l'hypoxie (Lanier et al, 1983 ; Wennergren et al, 1989 ; Sladek et al, 1993), l'anémie (Fagenholz et al, 1979) et une infection par le virus respiratoire syncytial (Pickens et al, 1989 ; Lindgren et al, 1992 ; Lindgren and Grogaard, 1996).

Malgré la grande importance clinique du CRL dans les premières semaines de vie, plusieurs inconnus persistent et la plupart des études sur modèle animal ne reflètent pas vraiment ce qui est vu en pratique. Premièrement, la majorité des études portant sur les CRL en période néonatale a été réalisée en utilisant l'eau distillée pour induire un CRL, alors que l'eau distillée n'est pas un stimulus mis en cause à cet âge en clinique. Ainsi, malgré la pertinence clinique évidente, peu d'études ont évalué l'effet d'une solution acide sur la muqueuse laryngée. Deuxièmement, en plus d'utiliser l'eau distillée, la majorité des études s'effectue avec un modèle animal anesthésié ou sédationné, et stimule la glotte *via* une trachéotomie stimulant ainsi la région sous-glottique de la trachée plutôt que la région supra-glottique. Finalement, la majorité de ces études possède un manque criant de standardisation et/ou évalue seulement un aspect des CRL. Dans ce contexte, mon laboratoire s'est engagé dans un vaste programme de recherche sur les CRL chez les agneaux nouveau-nés (à terme et prématurés). Mon projet de maîtrise s'intègre parfaitement dans ce programme dont l'objectif est d'étudier les CRL déclenchés par des solutions acides (acide chlorhydrique et acide citrique) en comparaison avec l'eau distillée (contrôle positif) et le sérum physiologique (salin : contrôle négatif) dans des conditions hautement standardisées. Afin d'être le plus près possible de la physiologie, cette étude sera réalisée chez un modèle animal sans sédation, soit l'agneau nouveau-né à terme, et en contrôlant les stades de conscience. Par la suite, une analyse fine sera réalisée en tenant compte de toutes les composantes des CRL. Cette étude n'est que le début d'un programme de recherche plus ambitieux qui permettra d'étudier l'influence de certains facteurs extérieurs sur les CRL.

Article

Laryngeal chemoreflexes induced by acid *vs* water *vs* saline in non-sedated newborn lambs during quiet sleep

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LARYNGEAL CHEMOREFLEXES INDUCED BY ACID VS. WATER VS. SALINE IN NON-SEDATED NEWBORN LAMBS DURING QUIET SLEEP

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ABSTRACT

Laryngeal chemoreflexes (LCR) are triggered by the contact between various liquids and the laryngeal mucosa. In the neonatal period, the immature LCR mainly consist of apnea and bradycardia, which at times can be life-threatening. The aim of the study was to assess the LCR induced in non-sedated, newborn full term lambs by several acid solutions, in comparison to distilled water and saline. Twelve lambs were instrumented for recording glottal adductor and diaphragm EMG, electroencephalogram, eye movements, heart rate, systemic arterial pressure and respiratory movements. LCR were induced during quiet sleep by the injection (0.5 ml) of saline, distilled water and two acid solutions (HCl and citric acid, pH 2, diluted in water and saline). A chronic supra-glottal catheter was used to inject the solutions in a random order. Distilled water and acid solutions identically induced no clinically significant cardiorespiratory responses. However, significant lower airway protective responses (swallowing, cough and arousal) were observed after distilled water, and especially acid solutions. In conclusion, LCR in full-term lambs, especially with acid solutions, are merely characterized by lower airway protective responses resembling the mature LCR reported in adult mammals.

Keywords : apnea, bradycardia, newborn, apparent life-threatening event

INTRODUCTION

The laryngeal chemoreflexes (LCR) are triggered by the contact between various liquids and receptors of the laryngeal mucosa in mammals. In a mature organism, liquids trigger highly protective reflexes aimed at preventing subglottal aspiration, mainly swallowing and cough. In the neonatal period, due to neurological immaturity, LCR include a vagal component with apnea, laryngospasm and bradycardia, and a sympathetic component with hypertension and redistribution of blood flow to vital organs, such as brain and heart (32). Clinically, it is widely acknowledged that the LCR can be triggered by acidity of gastro-esophageal reflux and can be responsible for apparent life threatening events and probably some cases of sudden infant death syndrome (24, 32, 34). While numerous studies have assessed LCR in response to various liquids in newborn mammals, the effects of acid solutions were studied much less often than water, despite the lesser relevance of the latter to the clinical situation. In addition, the majority of previous studies on LCR have been performed in anaesthetized or sedated animals, and/or aimed at stimulating the sub-glottal area, and/or suffer from a lack of standardization, and/or have been limited to only one aspect of the LCR, e.g. the apneic component. Within this context, we recently engaged in a research program on LCR using the newborn lamb (full-term and preterm) as the animal model, focusing on experiments relevant to the clinical situation. The aim of the present study was to assess extensively the various aspects of LCR induced by acid solutions (HCl and citric acid), in comparison with LCR induced by saline and water in standardized conditions. The latter include control of the state of alertness, avoidance of anesthesia/sedation, reproducibility of liquid injection just above the glottis, exhaustive analysis of various aspects of the LCR. In addition, it was understood from the beginning that expertise and knowledge gained from the present study would pave the way for future experiments on LCR mimicking pathological conditions in human infants.

MATERIALS AND METHODS

Animals

Experiments were performed in 12 lambs aged 2 - 3 days and weighing 4.6 ± 0.9Kg at the time of surgery. All lambs were born at term and housed with their mother in our animal quarters. The protocol of the study was approved by our institutional Ethics Committee for Animal Care and Experimentation.

Instrumentation of the lambs

Surgery was performed two days before the experiment under general anesthesia (2% isoflurane, 30% N_2O and 68% O_2). Atropine sulfate (0.1mg/kg), ketamine (10 mg/kg), and ketoprofen (3mg/kg) were injected intramuscularly before anesthesia. Antibiotics (0.05 ml/kg duplocillin and 5mg/kg gentamicin) were also injected intramuscularly before anesthesia and each day throughout the study. One lamb received one intramuscular injection of dexamethasone (0.5mg/kg) one day after surgery to decrease laryngeal inflammation manifested by weak bleating. Bipolar enameled chrome wire electrodes were inserted into the thyroarytenoid (TA, a glottal adductor) and diaphragm muscles for recording electrical activity (EMG) (14), together with custom-made electrodes for electroencephalogram (EEG) recordings (30). One cup electrode was also inserted under the scalp as a ground. Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. Correct positioning of electrodes in laryngeal and diaphragmatic muscles was always verified at autopsy. In addition, a supra-glottal catheter was inserted to allow injection of liquids onto the larynx. The catheter was adapted from an infusion catheter and placed by using a modified Seldinger technique with a metal guide wire inserted transcutaneously through the base of the epiglottis, as described previously (6). The tip of the catheter was positioned 5 to 7.5 mm above the anterior part of the glottis, while the external part of the catheter protruded 15-20 mm at the level of the anterosuperior aspect of the thyroid cartilage. The catheter was held in place by two rings, including one internal made of thick, rapid-drying adhesive glue, and one external made from the rubber end of a 3-ml syringe. Adequacy of the catheter positioning above the glottis was monitored throughout the insertion procedure by direct laryngoscopy, and was systematically checked during the autopsy. Finally, an arterial catheter was inserted into the brachial artery for recording systemic arterial pressure. All lambs were returned to their mother after arousal from anesthesia.

Additional instrumentation was performed right before the experiments, which were performed in non-sedated lambs. Nasal airflow was recorded using a thermocouple wire (iron/constantan, type J; Omega Engineering, Stamford, CO) glued to the side of one nostril. Two platinum needle electrodes (E2-12, Grass instrument company, Quincy, MA, USA) were placed subcutaneously into the outer upper region, and the inner lower region of the right eye socket for electrooculogram (EOG) recording. Three platinum needle electrodes (two on the foreleg root and one on the left hind leg root) were inserted subcutaneously for recording electrocardiogram (ECG). Thoracic and abdominal bands were placed for recording respiratory inductance plethysmography (Respitrace, NIMS, Miami Beach, FL, USA). Finally, an oximeter probe (8000R reflectance sensor, Nonin Medical, Plymouth, MN) was attached at the base of the tail to monitor pulse oximetry continuously.

Recording equipment

Leads from the EMG, EOG, EEG electrodes and the nasal thermocouple were connected to a transmitter attached to the lamb's back just prior to the experiment. The raw EMG, EOG and EEG signals and the nasal flow were transmitted by radiotelemetry using custom-made equipment (16). The raw EMG signals were moving time averaged (100 ms) by the acquisition software (Acknowledge Version 3.2, Biopac System Inc., Santa Barbara, California, USA). Systemic arterial pressure was obtained from the brachial catheter using a pressure transducer (Trantec model 60-800, American Edwards Laboratories, Santa Anna, CA, USA) and pressure monitor (model 78342A Hewlett Packard, Waltham, MA, USA). Lead 2 ECG was also obtained using this monitor. Thoracic and abdominal volume variations were qualitatively assessed using respiratory inductance plethysmography. Oxygenation was continuously monitored using our custom-made pulse oximeter built from a Nonin OEM with transmission by radiotelemetry (28). All parameters were continuously recorded using an Apple Macintosh microcomputer and the Acknowledge software. 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 and diaphragmatic EMG, ECG, EEG, EOG, nasal flow, sum of thoracic and abdominal movements, and arterial pressure. The lambs were comfortably positioned in a sling with loose restraints (Fig. 1).

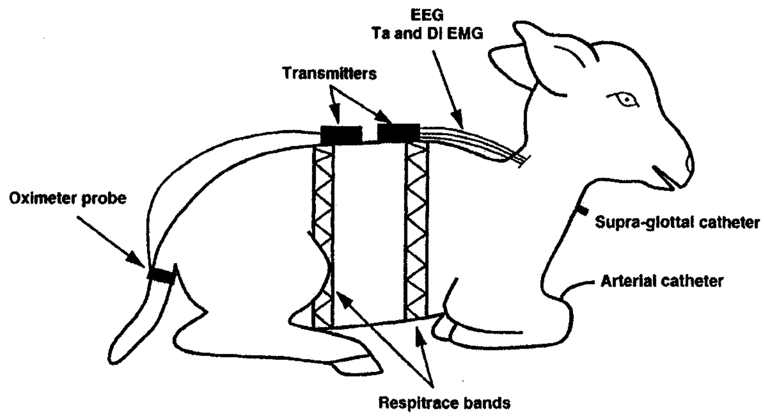

Figure 1: Schematic representation of the experimental design.

Ambient temperature was kept at 22°C, and humidity at 70% throughout the experimental days. Experiments were performed in non-sedated lambs over two consecutive days (*i.e.*, postnatal age 4 and 5 days or 5 and 6 days). For the first 8 lambs, each experimental day consisted of a random sequence of 8 laryngeal stimulations with 4 different solutions, totalizing 4 injections of each solution at the end of the 2 experimental days. Stimulations were performed only during quiet sleep by injecting 0.5 ml of hydrochloric acid (HCl) diluted in distilled water (pH =2), citric acid diluted in distilled water (pH = 2), distilled water and saline (0.9% NaCl, pH 5.5, used as control) through the supra-glottal catheter. In addition, 2 other solutions were also injected in the last 4 lambs [HCl diluted in saline, (pH = 2), and citric acid diluted in saline (pH = 2)], totalizing 12 injections by day of experimentation (table 1). Before injections, all solutions were warmed and maintained at the lamb's body temperature using a heating bath. Each animal was given at least 10 minutes of recovery time between 2 injections. Events such as agitation, cough and overt arousal / full awakening were noted by an observer throughout the recording sessions. Finally, 0.5 ml methylene blue was injected

Table 1: Characteristics of the solutions injected onto the larynx

	pH	Concentration in chloride ions (mmol/L)	Osmolarity (mosm/kg H_20)
Saline	6,79	181	326
Distilled water	6,77	0	0
HCl in H_20 (0.01 N)	1,9	11	17
Citric acid in H_20 (0.1 N)	1,9	19	126
HCl in saline (0.01 N)	2,2	181	295
Citric acid in saline (0.1 N)	1,9	233	511

through the supra-glottal catheter after completion of the experiments, to verify that the instillations were limited to the supra-glottal area (no staining beneath the glottis).

Data Analysis

The main objective of the study was to assess the LCR elicited by acid solutions (HCl and citric acid) in non-sedated lambs during quiet sleep, in comparison with saline (negative control) and distilled water (positive control). First, analysis of the cardiorespiratory responses to each laryngeal stimulus was performed as follows. The percentage of decrease in heart rate [$\%decHR = (HR_{BL} - HR_{min}) * 100/HR_{BL}$] was calculated, with HR_{BL} being the baseline heart rate (HR) value averaged on a 10 second period, within 30 seconds before challenge and HR_{min} the minimal HR value observed within 30 seconds after the challenge. Moreover, we noted whether a bradycardia was present, as defined by a %decHR greater than 30% (30). Similarly, the percentage of increase in mean arterial pressure [$\%incMAP = (MAP_{max} - MAP_{BL}) * 100/MAP_{BL}$] was calculated, with MAP_{BL} being the baseline mean arterial pressure (MAP) value averaged on the same 10 seconds period, within 30 seconds before challenge and MAP_{max} the maximal MAP value observed within 30 seconds after challenge. The percentage of decrease in respiratory rate [$\%decRR = (RR_{BL} - RR_{min}) * 100/RR_{BL}$] was also calculated, with RR_{BL} being the baseline respiratory rate (RR) averaged on the same 10 seconds period, within 30 seconds before challenge), and RR_{min} the minimal RR value observed within 30 seconds after challenge. The time durations between the moment of the stimulation and the moment of HR_{min}, MAP_{max} and RR_{min} occurrence (respectively HR_{min}, MAP_{max} and RR_{min} occurrence times) were measured for all stimulations. The presence of apneas (defined as at least 2 missed breaths, as referred to baseline breathing) was assessed within the 30 seconds interval following laryngeal stimulation. That allowed to calculate the percentage of stimulations with apnea(s) and the total, summed duration of apneas. Moreover, we noted whether apneas longer than 5 seconds were present. The respiratory LCR duration was measured as the time duration between the onset of the LCR and resumption of 3 consecutive breaths identical to baseline breathing (33). Aside from the cardiorespiratory responses, we counted the number of swallows (defined as a brisk, high amplitude and short duration TA EMG burst) (29) occurring within the 30 seconds interval following laryngeal stimulation. The time duration between the stimulation and the first swallow, and the time duration between the first and last swallow (total swallowing duration) were measured. In addition, total, summed duration of TA EMG (total TA EMG duration) was also calculated within the 30 seconds interval following laryngeal stimulation for each stimulus. The percentage of stimulations with coughing was also calculated. The presence of a cortical arousal was defined by the association of a change in EEG (decrease in amplitude + increase in frequency) for 3 seconds or more, with at least 2 of the following modifications: a 10% increase in heart rate, a change in respiration or a movement (13). A full awakening was quoted when the lamb was still awake after 1 minute (7). Percentage of stimulations triggering arousal and awakening were then calculated from these observations. Finally, total duration of LCR was measured as the time duration between the moment of the stimulation and the moment when respiratory rate, heart rate and mean arterial pressure were back to baseline values, with no swallowing or coughing for at least 10 seconds.

Statistical analysis: Measurements were first averaged in each lamb (mean of 4 injections for each stimulus) and then averaged for the 12 lambs as a whole. Values were then expressed as mean ± standard deviation. For all parameters, excepted % LCR with apnea(s), cough, arousal and awakening, differences in responses between the various laryngeal stimuli were assessed by one factor ANOVA for repeated measures, completed by Fisher's PLSD test, when indicated. For % LCR with apnea(s), cough, arousal, and awakening, statistical analyses were performed using the SAS software package; the effects of age and solution were tested by means of generalized estimating equation models, *i.e.*, generalized models with repeated measures (GENMOD procedure of SAS). The working correlation structure chosen was the exchangeable type. Since the response variable was a proportion, generalized linear model consisted in a logistic regression with a logit link function. For all parameters, a $p < 0.05$ was considered as statistically significant.

RESULTS

General results

A total of 203 laryngeal stimulations (46 saline, 45 distilled water, 48 HCl in water, 39 citric acid in water, 12 HCl in saline and 13 citric acid in saline) were performed in the 12 lambs during QS. Twenty-five stimulations (6 saline, 4 distilled water, 8 HCl in water, 5 citric acid in water, 1 HCl in saline and 1 citric acid in saline) could not be completely analyzed, due to either important agitation or coughing movements, or to loss of signal. Results are reported in table 2. All solutions, including saline, elicited a consistent sequence of events occurring within 10 seconds of laryngeal stimulation. Whatever the stimulus, arousal (when present) occurred immediately after liquid instillation, and swallowing activity (when present) began in the first 2 seconds after liquid instillation. Then, a decrease in RR and an increase in MAP were consistently observed, followed by a decrease in HR. Hence, occurrence time of HR_{min} was significantly delayed, as compared to occurrence time of RR_{min} and MAP_{max} ($p = 0.1$, RR_{min} *vs.* MAP_{max}; $p < 0.0001$, RR_{min} *vs.* HR_{min}; $p \leq 0.0001$, MAP_{max}, *vs.* HR_{min}). No differences in results were observed between day 4,5 and 6 of age.

Laryngeal chemoreflexes induced by saline and distilled water

The injection of 0.5ml saline onto the laryngeal mucosa elicited very mild cardiorespiratory responses (Fig. 2).

Table 2: Laryngeal chemoreflexes developing in response to various laryngeal stimuli in 12 lambs

	Saline	Distilled water	Hydrochloric acid in distilled water	Citric acid in distilled water
%dec HR	11.0 ± 4.3	15.5 ± 6.9 *	15.2 ± 6.5 *	18.7 ± 8.1 *,**,***
%inc MAP	8.4 ± 2.8	13.2 ± 3.6 *	14.4 ± 5 *	16.2 ± 5.8 *
%dec RR	30.7 ± 13.1	50.1 ± 12.15 *	45.4 ± 18.4 *	47.6 ± 11.8 *
HR_{min} occurrence time (s)	8.2 ± 2.2	6.3 ± 2.7	7.2 ± 3.4	7.1 ± 1.8
MAP_{max} occurrence time (s)	4.9 ± 2.4♣	4.6 ± 1.3♣	4.0 ± 1.4♣	5.2 ± 3.4
RR_{min} occurrence time (s)	4.1 ± 2.1♣	2.9 ± 1.1♣	4.5 ± 3.6♣	3.7 ± 2.3♣
% LCR with apnea(s)	14%	57%*,***,♥	36%*	42%*
Apnea duration (s)	0.6 ± 1	2.2 ± 1.5 *	1.9 ± 2.7 *	1.7 ± 1.5*
LCR respiratory duration (s)	3.0 ± 1.3	4.5 ± 1.6*	6.1 ± 3 *, **	6.3 ± 1.7 *, **
Swallow number	1.3 ± 0.8	2.3 ± 0.9*	3.3 ± 1.1 *	4.5 ± 1.7 *, **,***
First swallow occurrence time(s)	1.3 ± 0.8	1.9 ± 1.5	1.7 ± 0.7	2 ± 1.3
Total swallowing duration (s)	1.5 ± 1.4	4.0 ± 2.23*	5.3 ± 2.7*	8.0 ± 3*,**,***
Total TA EMG duration(s)	0.6 ± 0.2	1.5 ± 1 *	1.2 ± 0.8 *	1.7 ± 1.3 *
Cough	2%	10%	20%*	19%*
Total LCR duration (s)	3,4 ± 1,4	5,6 ± 1,8*	7,7 ± 3*,**	9,1 ± 2,4*,**
Arousal	59%	76%	87%*	97%*,**
Awakening	26%	39%*	52%*	72%*,**,***

LCR: laryngeal chemoreflexes. TA EMG duration: total duration of electrical activity of the thyroarytenoid muscle. HR: heart rate; MAP: mean arterial pressure; RR: respiratory rate. HR_{min}, MAP_{max} and RR_{min} occurrence time: time duration from injection to minimal HR value, or maximal MAP or RR value. %dec HR and RR: percentage decrease in HR or RR; %inc MAP: percentage increase in MAP. * $p < 0.05$ *vs.* saline. ** $p < 0.05$ *vs.* distilled water. *** $p < 0.05$ *vs.* hydrochloric acid. ♣ $p < 0.05$ *vs.* HR_{min} occurrence time. ♥ $p < 0.05$ *vs* citric acid.

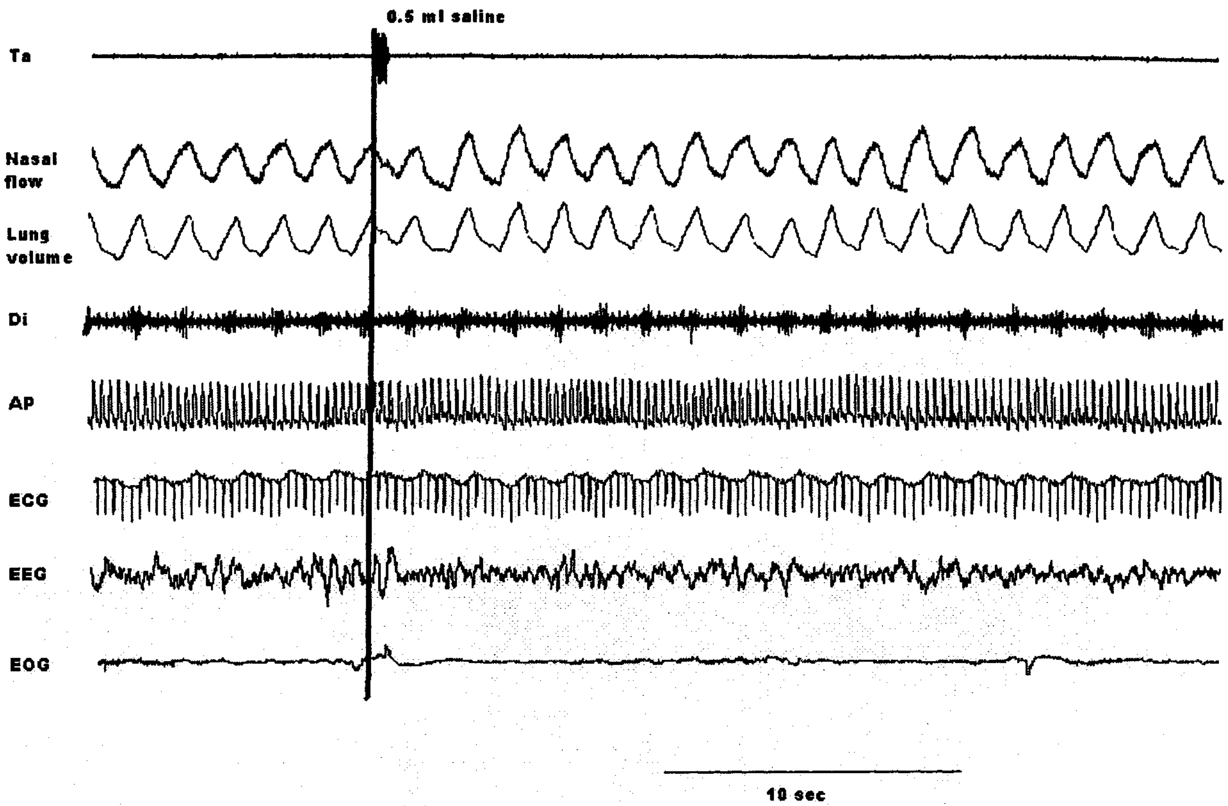

Figure 2: Laryngeal chemoreflexes in response to instillation of 0.5 mL saline onto the laryngeal mucosa in a non-sedated newborn lamb during quiet sleep. Ta; raw electrical activity of the thyroarytenoid muscle. Nasal flow: nasal airflow. Lung volume: sum signal of the respiratory inductance plethysmograph, allowing qualitative measurement of respiration (inspiration upwards). Di: raw electrical activity of the diaphragm. AP: systemic arterial pressure. ECG: electrocardiogram. EEG: electroencephalogram. EOG: electrooculogram.

Thyroarytenoid EMG activity, apneas, swallowing and coughing were virtually absent, and total LCR duration was 3.4 ± 1.4 s. However, injection of saline induced arousal from quiet sleep in 59% of the cases; among these, the lambs were fully awakened in 26% of the cases. *Distilled water* induced significantly more potent cardiorespiratory responses than saline (%dec HR: $p = 0.004$; %inc MAP: $p = 0.009$; %dec RR: $p < 0.0001$). Apneas were more prominent, and the respiratory LCR duration was significantly higher than with saline (total apnea duration: $p = 0.005$; % of stimulations with apnea: $p = 0.0003$; respiratory LCR duration: $p = 0.01$). While coughing was again a rare occurrence ($p = 0.25$ *vs.* saline), swallowing was significantly more frequent and lasted longer with water than saline ($p = 0.002$ and $p = 0.01$ respectively). Both total TA EMG duration and total LCR duration were higher than with saline ($p = 0.009$ and $p = 0.008$, respectively). Finally, while arousal from quiet sleep tended to occur more often with distilled water (76% of the cases) than saline, the difference was not significant ($p = 0.13$). Among these, lambs were fully awakened in 39% of the cases ($p = 0.04$ *vs.* saline). Overall, while distilled water induced (statistically) significantly more potent LCR than saline, it must be underlined that LCR still were mild.

<u>Laryngeal chemoreflexes induced by acid solutions</u>

Hydrochloric acid diluted in water elicited also mild cardiorespiratory responses, which were significantly more important than the ones induced by saline (%dec HR: $p = 0.007$; %inc MAP: $p = 0.001$; %dec RR: $p = 0.001$ *vs.* saline). Overall, cardiorespiratory responses were similar to those induced by distilled water (%dec HR: $p = 0.84$; %inc MAP: $p = 0.47$; %dec RR: $p = 0.26$). Similarly, citric acid diluted in water (Fig. 3)

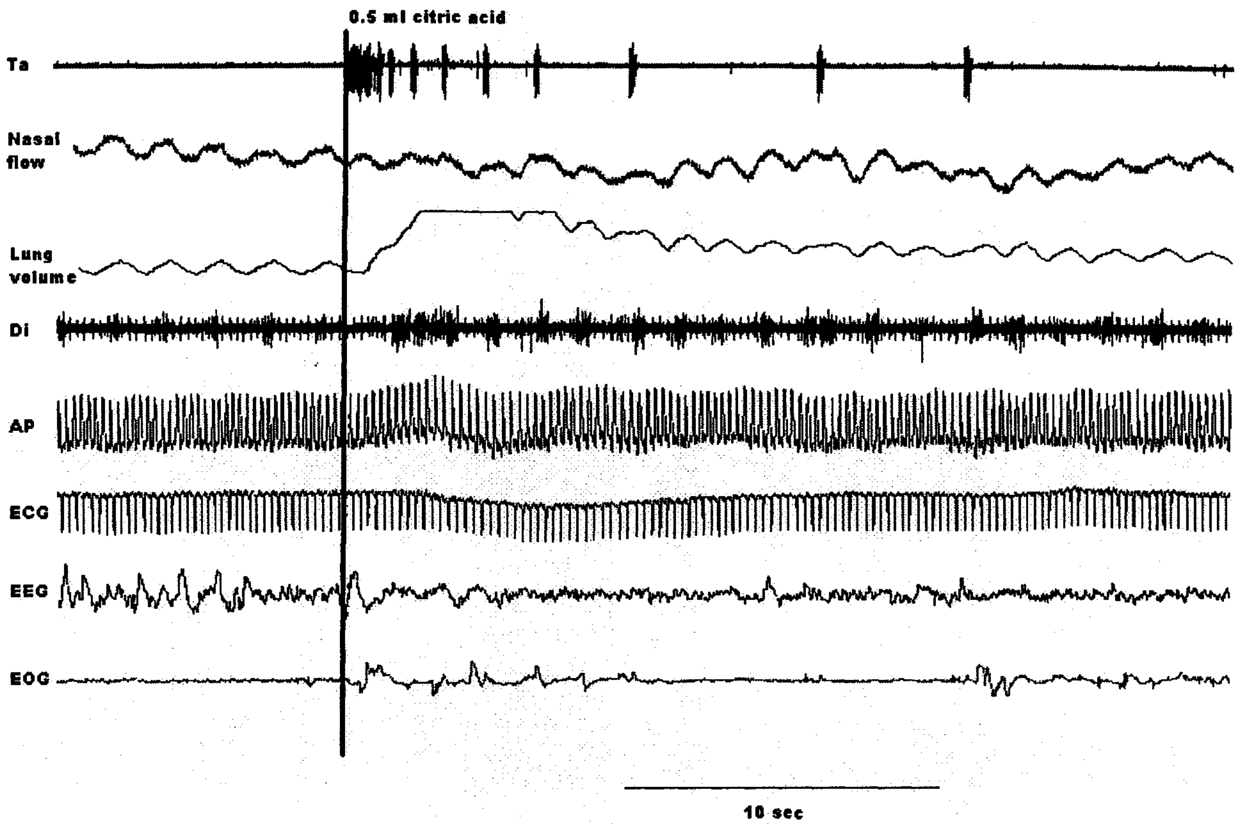

Figure 3: Laryngeal chemoreflexes in response to instillation of 0.5 mL citric acid diluted in water onto the laryngeal mucosa during quiet sleep (same lamb as in Fig 2). See Fig 2 for abbreviations.

induced more important cardiorespiratory responses than saline (%dec HR: $p < 0.0001$; %inc MAP: $p < 0.0001$; %dec RR: $p = 0.0003$). Of note, heart rate decreased significantly more following citric acid than distilled water ($p = 0.03$) and HCl ($p = 0.02$). Apneas were more prominent after acid solutions (diluted in distilled water) than saline, as assessed by the % of stimulations with apnea(s) (HCl: $p = 0.04$; citric acid: $p = 0.01$ *vs.* saline) and total apnea duration (HCl: $p = 0.02$; citric acid: $p = 0.04$ *vs.* saline). However, % of laryngeal stimulations with apnea(s) was higher with distilled water than either acid solution (distilled water *vs.* HCl: $p = 0.004$, and *vs.* citric acid: $p = 0.02$). LCR respiratory duration was greater following laryngeal stimulation by acid solutions than saline (HCl: $p < 0.0001$; citric acid: $p < 0.0001$) and distilled water (HCl: $p = 0.01$; citric acid: $p = 0.006$). Swallowing activity and coughing were significantly greater after both acid solutions than after saline (number of swallows, HCl: $p = 0.004$, citric acid: $p < 0.0001$; total swallowing duration, HCl: $p = 0.0003$, citric acid: $p < 0.0001$; cough bursts, HCl: $p = 0.02$, citric acid: $p = 0.05$). While citric acid in water induced significantly more swallows than distilled water ($p = 0.0004$) and HCl ($p = 0.02$), no significant difference was found between HCl and distilled water ($p = 0.5$). Also, while total swallowing duration was significantly greater with citric acid than both water and HCl ($p = 0.0002$ and $p = 0.008$ respectively), no significant difference was observed between HCl and water ($p = 0.16$). Total TA EMG duration was greater with acid solutions than saline (HCl: $p = 0.05$; citric acid: $p = 0.002$) and similar to distilled water (HCl: $p = 0.5$; citric acid: $p = 0.2$). Moreover, total duration of the LCR elicited by both acids diluted in water was significantly longer than the one elicited by distilled water (HCl: $p = 0.01$; citric acid: $p < 0.0001$) and saline (HCl: $p < 0.0001$; citric acid: $p < 0.0001$). Both HCl and citric acid diluted in water provoked arousal from quiet sleep more often than saline (HCl: $p = 0.002$; citric acid: $p = 0.0006$). In addition, while arousal following citric acid injection was more frequent than after distilled water ($p = 0.008$), this was not the case for HCl ($p = 0.06$). Finally, while awakening was observed more often with HCl and citric acid than saline (HCl: $p = 0.0009$; citric acid: $p = 0.0002$), citric acid induced awakening more often than distilled water ($p = 0.04$) and HCl ($p = 0.02$).

Acids diluted in saline. Results in the 4 lambs in which HCl and citric acid diluted in both distilled water and saline were tested are reported in table 3. Overall, identical cardiorespiratory responses were induced by HCl and citric acid, whether they were diluted in water or saline. Apneas, as assessed by the % of stimulations with apneas and total apnea duration, were identical whether acids were diluted in water or saline. LCR respiratory duration, however, was greater in HCl in water than in saline (HCl diluted in water, $p = 0.007$). While swallowing activity and total TA EMG were identical, whether acids were diluted in water or saline, total TA EMG duration was lower following HCl than citric acid. Cough bursts were mostly observed following both citric acid solutions, and at a lesser degree after distilled water and HCl diluted in water; cough was absent following HCl diluted in saline. Total LCR duration following citric acid diluted in saline was greater than after HCl diluted in water ($p = 0.02$) or saline ($p = 0.02$). Finally, arousal from quiet sleep was observed in more than 80% of the cases following acid injection, whether they were diluted in water or saline. However, full awakening appeared less frequent with HCl diluted in saline than with the other acid solutions.

Bradycardia, as defined by %decHR > 30%, was rarely observed in the healthy, full term lambs studied. Interestingly, the only lamb, who consistently presented bradycardia

Table 3: Comparison of laryngeal chemoreflexes elicited by acid solutions diluted in saline *vs.* in distilled water in 4 lambs

	Saline	Distilled water	Hydrochloric acid in distilled water	Hydrochloric acid in saline	Citric acid in distilled water	Citric acid in saline
%dec HR	9.9 ± 5.5	12.5 ± 6.42	12 ± 6	14.4 ± 8	17.2 ± 9	18.2 ± 12
%inc MAP	6.4 ± 2	14.4 ± 4.5	13.2 ± 5.8	8.9 ± 2.4	11.7 ± 3.3	13.6 ± 2.9
%dec RR	23.7 ± 10	45.4 ± 10	33.7 ± 11	30.7 ± 15	40.0 ± 7.3	37.4 ± 14.4
HR_{min} occurrence time (s)	8.3 ± 1	8.3 ± 4.3	6.1 ± 3.1	7 ± 1	7.1 ± 4.2	11.2 ± 3.3
MAP_{max} occurrence time (s)	3.9 ± 1.7***	3.6 ± 0.6	5 ± 1.1	8.5 ± 5.4	6.3 ± 2.9	5.8 ± 0.5***
RR_{min} occurrence time (s)	2.9 ± 0.4***	2.4 ± 0.9	3.3 ± 3.3	3 ± 1.9	5.7 ± 2.9	2.3 ± 0.5***
% LCR with apnea(s)	8%	36%	18%	17%	17%	27%
Apnea duration (s)	0.2 ± 0.5	1.9 ± 1.9	0.4 ± 0.5	0.7 ± 0.8	0.5 ± 0.7	0.6 ± 1.1
LCR respiratory duration (s)	2.2 ± 0.8	3.8 ± 0.8*	4.9 ± 1*,♣	2.8 ± 0.52	5.2 ± 0.9*,**,♣	5.4 ± 0.8*,♣,**
Swallow number	1.5 ± 1.3	2.4 ± 0.5	3.5 ± 0.4	2.6 ± 0.9	4.1 ± 1.3*	5.4 ± 1.7*,**,♣
First swallow occurrence time (s)	1.0 ± 1.1	2.2 ± 1.7	1.6 ± 0.6	1.5 ± 0.9	1.6 ± 0.4	1.7 ± 0.4
Total swallowing duration (s)	1.8 ± 1.7	2.3 ± 1	3.5 ± 1.2	4.3 ± 2.3	8.1 ± 4*,**,♦	11.6 ± 1.6*,**,♣,♦
Total TA EMG duration (s)	0.6 ± 0.2	1.8 ± 1.4	0.8 ± 0.3	0.7 ± 0.3	1.2 ± 0.4	1.4 ± 0.7
Cough	0%	15%	15%	0%	38%	33%
Total LCR duration (s)	3.1 ± 1.7	4.1 ± 0.42	5.3 ± 1.2	3.6 ± 1.2	8.4 ± 3.7*,**	8.4 ± 3.7*,**,♣,♦
Arousal	62%	92%	92%	82%	100%	100%
Awakening	43%	50%	73%	44%	85%	91%

*$p < 0.05$ *vs.* saline. ** $p < 0.05$ *vs.* distilled water. ♣ $p < 0.05$ *vs.* hydrochloric acid (in saline). ♦ $p < 0.05$ *vs* hydrochloric acid (in water).

*** $p < 0.05$ *vs* HR_{min} occurrence time following all stimuli (including saline), was later recognized as having abnormally increased heart rate variability during baseline (immaturity related-bradyarythmia). In the remaining 11 lambs, bradycardia was present only in 2 lambs following stimulation by citric acid. Moreover, apneas longer than 5 seconds were rare, occurring only after 2 stimulations with HCl diluted in water, 2 stimulations with distilled water and 3 stimulations with citric acid diluted in water.

DISCUSSION

The present study brings unique results on the laryngeal chemoreflexes induced in newborn lambs in highly standardized conditions. Overall, our findings show that in non-sedated, full-term lambs during quiet sleep, the cardiorespiratory components of the LCR were mild and similar for distilled water and acid solutions. However, acid solutions elicited more marked lower airway protective reflexes than distilled water, including swallowing, cough and arousal, suggesting that acid solutions (especially citric acid) are more potent stimuli than distilled water.

Uniqueness of our study in lambs

Review of the literature yields about fifty studies assessing LCR in newborn mammals, including human infants. From these studies, we were able to find only 10 studies, which were performed in the absence of anesthesia and sedation, either in lambs or piglets (8, 9, 18, 19, 20, 22), or in human infants (3, 23, 25, 26). However, two of those studies did not take the state of alertness into account (3, 9), and two others injected liquids through a tracheostomy towards the subglottal region (8, 18), what questions the specificity of the receptors (supraglottal, subglottal and/or tracheobronchial?) responsible for the reported LCR. In addition, despite the clinical relevance of acid solutions at this age, due to the possibility of acid gastro-esophageal reflux, only 2 of the above studies used acid solutions and compared it to saline and/or water (20, 22), while the others only used saline and/or water injection. Hence, to our knowledge, only one previous study performed in piglets (22) is comparable to our present study in lambs with regards to the absence of sedation, recording of the states of alertness, and comparison of an acid solution to both saline and water (22). However, further consideration of the latter study shows that our study design allows more complete assessment of LCR in the neonatal period. Indeed, while HCl was compared to both saline and water in only one piglet in the previous study (22), two different acid solutions were compared to both saline and water in all lambs of the present study. Also, we can claim better standardization due to greater control of the exact site of injection and mention of the solvent for acid solutions. In addition, though analysis of the LCR performed in the previous study was rather extensive (22), our analysis of LCR was extended even further by recording glottal adductor muscle activity, total duration of the LCR, and latencies of the various responses. Therefore, the present study provides the most complete analysis ever of LCR induced by 4 different stimuli (plus 2 different solvents for the 2 acids) in a non-sedated newborn mammal, in strictly controlled conditions. This hopefully will allow drawing powerful conclusions on LCR due to various liquid solutions in lambs, including the acid solutions, which are highly relevant to clinical conditions.

Laryngeal chemoreflexes induced by distilled water

Since the first observation by Johnson et al (1972) in anesthetized lambs, numerous studies have shown that the cardiorespiratory responses to application of water on the laryngeal mucosa are especially marked in newborn mammals (32, 34). Contrarily to water, saline was repeatedly shown to elicit very mild or no responses. Results of the present study confirm that saline induces virtually no cardiorespiratory responses. Moreover, present findings show that saline induces virtually no swallowing, no TA

EMG activity, cough or arousal. Conversely, our results confirm that distilled water induces cardiorespiratory responses significantly more marked than saline, including a decrease in RR (sometimes up to short apnea), a decrease in HR and an increase in MAP. In addition, TA EMG activity after water was significantly greater than after saline. However, the cardiorespiratory responses we observed with water were less pronounced that the ones traditionally reported in the literature. For example, distilled water was reported to induce prolonged apnea followed by gasping and sometimes death in anaesthetized piglets (15, 4). Regarding cardiovascular responses, water was reported to induce a 44% decrease in HR and a 31% increase in MAP in non-sedated, awake lambs (8), which represents alterations well above the ones we observed in the present study. Discrepancies between those previous results and ours regarding cardiorespiratory responses are probably mainly related to the use of anesthesia and/or the greater volume of water injected (15 ml *vs.* 0.5 ml) and/or the site of injection (sub-glottal *vs.* supra-glottal) in previous studies. Accordingly, results in non-sedated piglets using a volume of water closer to the one we used yielded similarly mild cardiorespiratory responses (22). Finally, our results showing that swallowing and arousal were more often observed after water than saline is in agreement with previous studies (3, 22).

<u>Laryngeal chemoreflexes induced by acid solutions</u>

Overall, apart from the more frequent observation of short apneas after distilled water than acid solutions, the cardiorespiratory responses elicited by distilled water and HCl were identical, which is in agreement with results in piglets (22). As for citric acid, it induced a significantly greater decrease in HR than both distilled water and HCl. The reason for this difference is unknown. Conversely, protective lower airway mechanisms were more marked after acid solutions than distilled water. Indeed, arousal and full awakening, cough and total LCR duration were significantly more marked with acid solutions than with distilled water and saline. Conversely to our present results, swallowing activity was reported to be more prominent with water than HCl (pH 2) in piglets during natural sleep (22). However, our results are in agreement with another previous study in adult rats, showing that swallowing is more marked following pharyngeal infusion of citric acid than distilled water (12). This was interpreted as being related to the sour taste of citric acid, what our study do not allow to comment further.

While study of the solutions composed of acids diluted in water might represent a maximal stimulus for the LCR (stimulation by both water and acid), comparison to responses elicited by acids diluted in saline allows to explore the LCR triggered by acid stimulus alone. Analysis of the results suggests that either saline or water solution grossly triggers identical LCR, what cannot be explained by our study. Interestingly, for unknown reasons, LCR elicited by citric acid solutions appear to be more marked than LCR elicited by HCl.

Differences between responses to distilled water and acid solutions are probably mainly related to stimulation of different laryngeal receptors. Boggs (2) initially showed that the cardiorespiratory responses induced by contact of distilled water with the laryngeal mucosa are related to low chloride concentration. Since then, two types of water receptors have been described in the laryngeal mucosa, including a short latency receptor, which responds to low chloride concentration (rapidly adapting receptor), and a long latency receptor, which responds to hypo-osmolality (1). Conversely, protons have been shown to stimulate different receptors, namely the acid sensing ion channels (ASIC) and the vanilloid receptor (VR), which are present on C and Aδ fibers (11, 17, 27,). As for the larynx, C fiber endings have been shown to be sensitive to citric acid in adult guinea pigs (5), but insensitive to water in adult dogs (21) and guinea-pigs (5). Our previous results in lambs suggest that laryngeal C fibers are already functional in the neonatal period, and insensitive to water also (31).

In conclusion, full-term lambs appear to have mature LCR, *i.e.*, characterized by lower airway protective mechanisms and absence of clinically significant apnea-bradycardia. Reasons for the apparent discrepancy with most previous studies in newborn mammals are likely mainly related to experimental conditions, which are farer from clinical conditions. Our observations of very mild cardiorespiratory responses in full term lambs, but of clinically significant apnea-bradycardia after acid solutions in preterm lambs (preliminary observations) is in agreement with clinical observations in full term and preterm human infants. Further studies will have to delineate further clinically relevant conditions, which exacerbate the LCR, and to study the neuronal mechanisms responsible for LCR triggered by acid solutions, due to the clinical relevance of the later.

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REFERENCES

1. **Anderson JW, Sant'Ambrogio FB, Mathew OP, and Sant'Ambrogio G**. Water-responsive laryngeal receptors in the dog are not specialized endings. *Respir Physiol* 79: 33-43, 1990.

2. **Boggs DF, and Bartlett D Jr**. Chemical specificity of a laryngeal apneic reflex in puppies. *J Appl Physiol* 53: 455-462, 1982.

3. **Davies AM, Koenig JS, and Thach BT**. Upper airway chemoreflex responses to saline and water in preterm infants. *J Appl Physiol* 64: 1412-1420, 1988.

4. **Downing SE, and Lee JC**. Laryngeal chemosensitivity: a possible mechanism for sudden infant death. *Pediatrics* 55: 640-649, 1975.

5. **Forsberg K, Karlsson JA, Theodorsson E, and Lundberg JM.** Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea-pig. *Pulm Pharmacol* 1: 33-39, 1988.

6. **Fortier PH, Reix P, Arsenault J, Dorion D, and Praud JP**. Active upper airway closure during induced central apneas in lambs is complete at the laryngeal level only. *J Appl Physiol* 95: 97-103, 2003.

7. **Goto K, Mirmiran M, Adams MM, Longford RV, Baldwin RB, Boeddiker MA, and Ariagno RL**. More awakenings and heart rate variability during supine sleep in preterm infants. *Pediatrics* 103: 603-609, 1999.

8. **Grogaard J, Lindstrom DP, Stahlman MT, Marchal F, and Sundell H**. The cardiovascular response to laryngeal water administration in young lambs. *J Dev Physiol* 4: 353-370, 1982.

9. **Harned HS, Myracle J, and Ferreiro J.** Respiratory suppression and swallowing from introduction of fluids into the laryngeal region of the lamb. *Pediatr Res* 12: 1003-1009, 1978.

10. **Johnson P, Dawes DS, and Robinson JS.** Maintenance of breathing in newborn lamb. *Arch Dis Child* 47:151, 1972.

11. **Julius D, and Basbaum AI.** Molecular mechanisms of nociception. *Nature* 413(6852): 203-210, 2001.

12. **Kajii Y, Shingai T, Kitagawa J, Takahashi Y, Taguchi Y, Noda T, and Yamada Y.** Sour taste stimulation facilitates reflex swallowing from the pharynx and larynx in the rat. *Physiol Behav* 77: 321-325, 2002.

13. **Kato I, Franco P, Groswasser J, Scaillet S, Kelmanson I, Togari H, and Kahn A.** Incomplete arousal processes in infants who were victims of sudden death. *Am J Respir Crit Care Med* 168: 1298-1303, 2003.

14. **Kianicka I, Leroux JF, and Praud JP.** Thyroarytenoid muscle activity during hypocapnic central apneas in awake nonsedated lambs. *J Appl Physiol* 76: 1262-1268, 1994.

15. **Lee JC, Stoll BJ, and Downing SE.** Properties of the laryngeal chemoreflex in neonatal piglets. *Am J Physiol* 233: R30-R36, 1977.

16. **Letourneau P, Dumont S, Kianicka I, Diaz V, Dorion D, Drolet R, and Praud JP.** Radiotelemetry system for apnea study in lambs. *Respir Physiol* 116: 85-93, 1999.

17. **Mamet J, and Voilley N.** Les canaux sodiques activés par l'acidification extracellulaire et la douleur inflammatoire. *Medecine Sciences* 18: 889-895, 2002.

18. **Marchal F, Corke BC, and Sundell H.** Reflex apnea from laryngeal chemo-stimulation in the sleeping premature newborn lamb. *Pediatr Res* 16: 621-627, 1982.

19. **Marchal F, Crance JP, and Arnould P.** Ventilatory and waking responses to laryngeal stimulation in sleeping mature lambs. *Respir Physiol* 63: 31-41, 1986.

20. **McKelvey GM, Post EJ, Wood AK, and Jeffery HE.** Airway protection following simulated gastro-oesophageal reflux in sedated and sleeping neonatal piglets during active sleep. *Clin Exp Pharmacol Physiol* 28: 533-539, 2001.

21. **Mutoh T, Kanamaru A, Kojima K, Nishimura R, Sasaki N, and Tsubone H.** Effects of perineural capsaicin treatment on cardiopulmonary reflexes elicited by laryngeal instillations of capsaicin and distilled water in sevoflurane-anesthetized dogs. *J Vet Med Sci* 62: 665-668, 2000.

22. **Page M, Jeffery HE, Marks V, Post EJ, and Wood AK.** Mechanisms of airway protection after pharyngeal fluid infusion in healthy sleeping piglets. *J Appl Physiol* 78: 1942-1949, 1995.

23. **Page M, and Jeffery HE.** Airway protection in sleeping infants in response to pharyngeal fluid stimulation in the supine position. *Pediatr Res* 44: 691-698, 1998.

24. **Page M, and Jeffery H.** The role of gastro-oesophageal reflux in the aetiology of SIDS. *Early Hum Dev* 59: 127-149, 2000.

25. **Perkett EA, and Vaughan RL.** Evidence for a laryngeal chemoreflex in some human preterm infants. *Acta Paediatr Scand* 71: 969-972, 1982.

26. **Pickens DL, Schefft G, and Thach BT.** Prolonged apnea associated with upper airway protective reflexes in apnea of prematurity. *Am Rev Respir Dis* 137: 113-118, 1988.

27. **Reeh PW, and Kress M**. Molecular physiology of proton transduction in nociceptors. *Curr Opin Pharmacol* 1: 45-51, 2001.

28. **Reix P, Arsenault J, Dome V, Fortier PH, Lafond-Rouillard J, Moreau-Bussiere F, Dorion D, and Praud JP**. Active glottal closure during central apneas limits oxygen desaturation in premature lambs. *J Appl Physiol* 94: 1949-1954, 2003.

29. **Reix P, Fortier PH, Niyonsenga T, Arsenault J, Letourneau P, and Praud JP**. Non nutritive swallowing and respiration coordination in full-term newborn lambs. *Respir Physiol Neurobiol* 134: 209-218, 2003.

30. **Renolleau S, Letourneau P, Niyonsenga T, and Praud JP**. Thyroarytenoid muscle electrical activity during spontaneous apneas in preterm lambs. *Am J Respir Crit Care Med* 159: 1396-1404, 1999.

31. **Roulier S, Arsenault J, Reix P, Dorion D, and Praud JP**. Effects of C fiber blockage on cardiorespiratory responses to laryngeal stimulation in conscious lambs. *Respir Physiol Neurobiol* 136: 13-23, 2003.

32. **Thach BT**. Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to adult life. *Am J Med* 111: 69S-77S, 2001.

33. **Van Der Velde L, Curran AK, Filiano JJ, Darnall RA, Bartlett D Jr, and Leiter JC**. Prolongation of the laryngeal chemoreflex after inhibition of the rostral ventral medulla in piglets: a role in SIDS? *J Appl Physiol* 94: 1883-1895, 2003.

34. **Wetmore RF**. Effects of acid on the larynx of the maturing rabbit and their possible significance to the sudden infant death syndrome. *Laryngoscope* 103: 1242-1254, 1993.

Discussion

Cette étude effectuée chez un modèle d'agneau à terme sans sédation dans des conditions standardisées apporte des résultats uniques sur les CRL et plus particulièrement sur les CRL déclenchés par l'acide. Les composantes cardiaques et respiratoires des CRL obtenues suite aux stimulations laryngées par les solutions acides et l'eau distillée sont statistiquement significativement différentes de celles obtenues avec le salin (témoin négatif), mais d'importance clinique négligeable. Ces réponses cardiorespiratoires minimes sont similaires, peu importe que la stimulation soit de l'eau distillée ou des solutions acides. Cependant, une analyse plus approfondie démontre que les solutions acides, plus particulièrement l'acide citrique, déclenchent des réflexes de protection des VAI plus marqués que l'eau distillée. Déglutitions, toux et réaction d'éveil sont ainsi fréquemment observées suite à l'injection des solutions acides suggérant que les solutions acides sont de plus puissants stimuli.

Une revue exhaustive de la littérature a permis de constater qu'il existe seulement une cinquantaine d'étude portant sur les CRL chez les mammifères nouveau-nés, incluant les nouveau-nés humains. De ce nombre, seulement dix études ont été effectuées en l'absence d'anesthésie ou de sédation chez l'agneau et le cochonnet nouveau-nés (Harned et al, 1978 ; Grogaard et al, 1982 ; Marchal et al, 1982 ; Marchal et al, 1986 ; Page et al, 1995 ; McKelvey et al, 2001) et les nouveau-nés humains (Perkett et al, 1982 ; Davies et al, 1988 ; Pickens et al, 1988 ; Page et al, 1998). Parmi ces dix études, deux ne tiennent pas compte des stades de conscience (Harned et al, 1978 ; Davies et al, 1988) et deux injectent leur solution *via* une trachéotomie (Grogaard et al, 1982 ; Marchal et al, 1982) nous questionnant ainsi sur la spécificité des récepteurs (supra-glottiques *vs.* sub-glottiques) responsables des CRL rapportés dans ces études. Malgré la pertinence clinique évidente de l'utilisation de solutions acides pour induire les CRL, la plupart des études utilise l'eau distillée, et seulement deux études comparent les réponses induites par les solutions acides au salin ou à l'eau distillée (Page et al, 1995 ; McKelvey et al, 2001). Finalement, parmi la cinquantaine d'études, une seule effectuée chez les cochonnets nouveau-nés est comparable à la nôtre (Page et al, 1995). Cependant, notre étude est plus complète, car elle apporte des résultats uniques sur les CRL déclenchés par six stimuli différents (salin, eau distillée, acide chlorhydrique en solution saline et aqueuse, acide citrique en solution saline et aqueuse) chez l'agneau nouveau-né sans sédation dans des conditions très standardisées et avec une analyse exhaustive.

Chémoréflexes laryngés déclenchés par l'eau distillée

Depuis la première observation du phénomène par Jonhson et al (1972) chez les agneaux anesthésiés, plusieurs études ont par la suite démontré que l'application d'eau distillée sur la muqueuse laryngée, contrairement au salin, induit des réponses cardiorespiratoires importantes (Wetmore, 1993 ; Thach, 2001). Dans notre étude, le salin induit des réponses comparables à celles rapportées dans la littérature c'est-à-dire très peu de réponses cardiorespiratoires, peu de déglutitions, peu de toux et finalement peu de réactions d'éveil. Contrairement au salin, l'eau distillée induit des réponses cardiorespiratoires significativement plus marquées, incluant une diminution de la Fr (parfois avec présence de courte apnée), une diminution de la Fc, une augmentation de la PA moyenne et une activité du Ta plus importante. Par contre, on constate que mon modèle semble induire des réponses cardiorespiratoires beaucoup moins prononcées que celles rapportées dans la littérature. Des apnées prolongées voire même fatales ont ainsi souvent été observées chez les cochonnets anesthésiés (Downing et al, 1975 ; Lee et al, 1977). Grogaard et al (1982) a démontré chez un modèle d'agneau éveillé que les réponses cardiovasculaires obtenues suite à une stimulation laryngée par l'eau distillée sont majeures, (diminution de la Fc de 44% et augmentation de la PA de 31%). Un bémol doit être par contre apporté à ces résultats car ils sont obtenus avec des modèles anesthésiés et un volume d'eau distillée beaucoup plus grand (15 ml *vs* 0.5 ml). La seule étude comparable à la nôtre démontre, elle aussi, des réponses significativement plus importantes avec l'eau distillée que le salin, mais malgré tout des réponses cardiorespiratoires minimes même avec l'eau distillée. Finalement, en accord avec d'autres études (Davies et al, 1988 ; Page et al, 1995), déglutitions, réaction d'éveil et éveil sont plus souvent observés après l'application d'eau distillée que de salin.

Chémoréflexes laryngés déclenchés par les solutions acides

Mis à part que des courtes apnées ont été observées plus souvent avec l'eau distillée qu'avec les solutions acides, les réponses cardiorespiratoires déclenchées par l'eau et l'acide chlorhydrique étaient identiques, en accord avec les résultats obtenus chez les cochonnets (Page et al, 1995). A noter que pour une raison encore inconnue, l'acide citrique a induit une diminution de la Fc significativement plus importante que l'eau distillée et le HCl. Contrairement à nos résultats, Page et al (1995) a rapporté que les déglutitions étaient plus fréquentes avec l'eau distillée comparativement à l'HCl (pH 2). Cependant, il faut noter que la fiabilité de leur conclusion est limitée par le fait que seul un cochonnet a reçu toutes les solutions à l'étude. Par contre, une autre étude a montré que les déglutitions étaient plus marquées suivant une infusion au niveau du pharynx avec l'acide citrique comparativement à l'eau distillée, ce qui était attribué au caractère acide lui-même (Kajii et al, 2002). Pour ce qui a trait à la réaction d'éveil, nos résultats démontrent qu'elle est plus fréquente suivant une infusion avec HCl comparativement à l'eau exactement comme démontré par Page et al (1995).

Eau distillée *vs* solutions acides

Même si les réponses cardiorespiratoires sont identiques, déglutitions, toux, réaction d'éveil et réveil complet sont plus souvent observés avec les solutions acides que l'eau distillée. Ainsi, les réponses différentes suggèrent la possibilité de mécanismes différents et spécifiques pour chaque sorte de stimulation. L'hypothèse la plus plausible est que l'eau distillée et l'acide stimulent des récepteurs différents au niveau de la muqueuse laryngée.

Eau distillée

L'eau distillée appliquée au niveau de la muqueuse laryngée stimule ce qu'on appelle des récepteurs à l'eau. Bogg and Bartlett (1982) a démontré que ces récepteurs sont activés par une faible concentration en ions chlorures de façon dose dépendante, les réponses étant d'autant plus importantes que la concentration en ion chlorure est faible. Depuis, d'autres équipes ont démontré que la muqueuse laryngée contient deux types de récepteur à l'eau, répondant chacun à une sorte de stimulation. Un type de récepteur, de courte latence, est stimulé par une faible concentration en ions chlorures (récepteur à adaptation rapide) tandis que l'autre, de longue latence, répond à l'hypo-osmolarité (récepteur à adaptation lente) (Anderson et al; 1990).

Solutions acides

Les protons stimulent, eux aussi, deux types de récepteur soit les canaux sensibles aux pH (ASIC, pour *acid sensing ion channels*) et les récepteurs ionotropiques des vanilloïdes (VR, comme le récepteur de la capsaïcine VR1). Ces deux types de récepteur laissent entrer des cations dans la cellule sous l'action des protons. Il est démontré que ces deux types de récepteur sont retrouvés sur ce que l'on appelle les fibres sensibles à la capsaïcine, soit les fibres C et les fibres Aδ (Reech et al, 2001; Julius et al, 2001; Mamet et al, 2002). En période néonatale, la muqueuse laryngée contient une quantité importante de ces fibres (Miller et al, 1976 ; Chung et al 1993) qui sont, en plus, déjà fonctionnelles à cette période (Roulier et al, 2003). Les fibres C laryngées ont fait l'objet de plusieurs études démontrant ainsi que ces fibres sont sensibles à de nombreuses substances dont l'acide citrique (Forsberg et al, 1988), mais insensibles à d'autres dont l'eau distillée (Forsberg et al, 1988 ; Mutoh et al, 2000 ; Roulier et al, 2003). Les fibres C sont des nocicepteurs polymodaux impliquées principalement dans la perception des stimuli douloureux qu'ils soient mécaniques, thermiques ou chimiques. Par conséquent, les solutions acides stimuleraient des récepteurs présents sur des fibres à la douleur tandis que l'eau distillée stimulerait des récepteurs présents sur des fibres différentes de celles de la douleur. La prédominance des mécanismes de protection des VAI avec les solutions acides pourraient s'expliquer par le fait que ces solutions sont transmises comme étant des stimulations douloureuses. Les quelques différences observées entre HCl et l'acide citrique peuvent être attribuées, selon moi, au fait que les récepteurs ASIC et VR sont activés à des degrés différents par ces deux acides, l'acide citrique étant probablement un plus puissant activateur que ne l'est HCl.

Les huit premiers agneaux ont reçu des solutions acides mais diluées dans l'eau distillée. Ceci ne nous permettait pas de tester l'effet spécifiques des protons, car ces solutions ont une concentration en ions chlorures très faible. Bogg and Bartlett (1982) ont démontré qu'une concentration en ions chlorures inférieure à 80-100mM déclenche des apnées. Par conséquent, il est impossible d'assurer que les CRL obtenus suite à la stimulation laryngée avec ces solutions acides sont reliées exclusivement aux protons. Afin de vérifier les effets spécifiques aux protons, les quatre derniers agneaux ont reçu de l'HCl et de l'acide citrique en solution aqueuse et en solution saline. On a ainsi pu constater que les deux types de solutions d'acide induisent des réponses similaires. Cette étude a donc permis de montrer que les protons, à eux seuls, induisent des réponses cardiorespiratoires et des mécanismes de protection des VAI. Ces résultats sont très intéressants pour leurs implications cliniques pour les nombreux nouveau-nés et nourrissons qui ont des reflux gastro-œsophagiens acides. De plus, l'observation que l'addition du stimulus « faible concentration en ions chlorures » (solution aqueuse d'acide) au stimulus « protons » (solution saline d'acide) n'entraîne pas vraiment de CRL plus marqué est intéressant sur le plan physiologique. Cependant, il reste maintenant à confirmer les hypothèses sur les mécanismes en cause dans les CRL déclenché par chaque stimulus.

<u>Maturation des réflexes</u>

Chez les organismes immatures, les CRL associent, en autre, apnée, bradycardie, laryngospasme. Une maturation post-natale remplace ces réflexes délétères par des mécanismes de défense incluant déglutitions, toux et éveil. Les agneaux nés à terme âgés de 4 à 6 jours utilisés dans notre étude semblent donc avoir déjà subi un certain processus de maturation car les réponses cardiorespiratoires obtenues étaient pour ainsi dire minimes et les réflexes de protection des VAI prédominants. Suite à cette étude, il semble ainsi évident que les agneaux nouveau-nés à terme étudiés sans sédation ont des réflexes de type mature. En accord avec ces résultats, il est intéressant de noter que les réponses cardiorespiratoires responsables d'apnées et de bradycardies chez les nouveau-nés humains sont généralement retrouvées chez des nourrissons présentant des signes d'immaturité rhombencéphalique, tels les nouveau-nés prématurés, et/ou chez des nourrissons souffrant de conditions exacerbant les CRL (laryngite de reflux ou infection par le virus respiratoire syncytial, par exemple). Cette concordance nous donne à penser que le modèle ovin est pertinent pour poursuivre l'étude des CRL dans des situations tentant de reproduire les situations cliniques sus-citées.

Conclusion

Cette étude volontairement descriptive a permis pour la première fois de caractériser très précisément les CRL déclenchés par des solutions d'acide déposées directement sur la muqueuse laryngée chez des nouveau-nés à terme et en santé. Elle nous a permis de démontrer que les agneaux nés à terme et en santé ont des CRL caractérisés par des mécanismes de protection des VAI plutôt que par des apnées et des bradycardies cliniquement significatives.

Quelles sont donc les situations où l'on peut retrouver des CRL immatures ? Notre hypothèse est qu'il s'agit, en autre, de la prématurité, des situations où une inflammation laryngée est présente (infection virale des voies aériennes supérieures, laryngite de reflux) et des complications liées à la fumée secondaire. Par conséquent, le but de mes projets de recherche de doctorat sera d'étudier l'influence de ces facteurs extérieurs sur les CRL, espérant ainsi un jour comprendre et même éviter certains cas de mort subite du nourrisson, qui fait hélas encore trop de jeunes victimes chaque année.

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Références

Anderson JW, Sant'Ambrogio FB, Mathew OP, and Sant'Ambrogio G. Water-responsive laryngeal receptors in the dog are not specialized endings. *Respir Physiol* 79: 33-43, 1990.

Boggs DF, and Bartlett D Jr. Chemical specificity of a laryngeal apneic reflex in puppies. *J Appl Physiol* 53: 455-462, 1982.

Chan K, Kallama LK, Day L, Ogundipe A, and Ross MG. Ovine fetal laryngeal chemoreflex thresholds and respiratory effets. *Otolaryngol Head Neck Surg* 116: 91-96, 1997.

Davies AM, Koenig JS, and Thach BT. Upper airway chemoreflex responses to saline and water in preterm infants. *J Appl Physiol* 64: 1412-1420, 1988.

Downing SE, and Lee JC. Laryngeal chemosensitivity: a possible mechanism for sudden infant death. *Pediatrics* 55: 640-649, 1975.

Fagenholz SA, Lee JC, and Downing SE. Association of anemia with reduced central respiratory drive in the piglet. *Yale J Biol Me* 52: 263-270, 1979.

Forsberg K, Karlsson JA, Theodorsson E, and Lundberg JM. Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea-pig. *Pulm Pharmacol* 1: 33-39, 1988.

Fortier PH, Reix P, Arsenault J, Dorion D, and Praud JP. Active upper airway closure during induced central apneas in lambs is complete at the laryngeal level only. *J Appl Physiol* 95: 97-103, 2003.

Goto K, Mirmiran M, Adams MM, Longford RV, Baldwin RB, Boeddiker MA, and Ariagno RL. More awakenings and heart rate variability during supine sleep in preterm infants. *Pediatrics* 103: 603-609, 1999.

Grogaard J, Lindstrom DP, Stahlman MT, Marchal F, and Sundell H. The cardiovascular response to laryngeal water administration in young lambs. *J Dev Physiol* 4: 353-370, 1982.

Harned HS, Myracle J, and Ferreiro J. Respiratory suppression and swallowing from introduction of fluids into the laryngeal region of the lamb. *Pediatr Res* 12: 1003-1009, 1978.

Johnson P, Dawes DS, and Robinson JS. Maintenance of breathing in newborn lamb. *Arch Dis Child* 47:151, 1972.

Julius D, and Basbaum AI. Molecular mechanisms of nociception. *Nature* 413(6852): 203-210, 2001.

Kajii Y, Shingai T, Kitagawa J, Takahashi Y, Taguchi Y, Noda T, and Yamada Y. Sour taste stimulation facilitates reflex swallowing from the pharynx and larynx in the rat. *Physiol Behav* 77: 321-325, 2002.

Kato I, Franco P, Groswasser J, Scaillet S, Kelmanson I, Togari H, and Kahn A. Incomplete arousal processes in infants who were victims of sudden death. *Am J Respir Crit Care Med* 168: 1298-1303, 2003.

Kianicka I, Leroux JF, and Praud JP. Thyroarytenoid muscle activity during hypocapnic central apneas in awake nonsedated lambs. *J Appl Physiol* 76: 1262-1268, 1994.

Lanier B, Richardson MA, and Cummings C. Effect of hypoxia on laryngeal reflex apnea: implication for sudden infant death. *Otolaryngol Head Neck Surg* 91: 597-604, 1983.

Lee JC, Stoll BJ, and Downing SE. Properties of the laryngeal chemoreflex in neonatal piglets. *Am J Physiol* 233: R30-R36, 1977.

Letourneau P, Dumont S, Kianicka I, Diaz V, Dorion D, Drolet R, and Praud JP. Radiotelemetry system for apnea study in lambs. *Respir Physiol* 116: 85-93, 1999.

Lindgren C, Jing L, Graham B, et al. Respiratory syncytial virus infection reinforces reflex apnea in young lambs. *Pediatr Res* 31: 381-385, 1992.

Lindgren C, and Grogaard J. Reflex apnoea response and inflammatory mediators in infants with respiratory tract infection. *Acta Paediatr* 85: 798-803, 1996.

Mamet J, and Voilley N. Les canaux sodiques activés par l'acidification extracellulaire et la douleur inflammatoire. *Medecine Sciences* 18: 889-895, 2002.

Marchal F, Corke BC, and Sundell H. Reflex apnea from laryngeal chemo-stimulation in the sleeping premature newborn lamb. *Pediatr Res* 16: 621-627, 1982.

Marchal F, Crance JP, and Arnould P. Ventilatory and waking responses to laryngeal stimulation in sleeping mature lambs. *Respir Physiol* 63: 31-41, 1986.

McKelvey GM, Post EJ, Wood AK, and Jeffery HE. Airway protection following simulated gastro-oesophageal reflux in sedated and sleeping neonatal piglets during active sleep. *Clin Exp Pharmacol Physiol* 28: 533-539, 2001.

Mutoh T, Kanamaru A, Kojima K, Nishimura R, Sasaki N, and Tsubone H. Effects of perineural capsaicin treatment on cardiopulmonary reflexes elicited by laryngeal instillations of capsaicin and distilled water in sevoflurane-anesthetized dogs. *J Vet Med Sci* 62: 665-668, 2000.

Page M, Jeffery HE, Marks V, Post EJ, and Wood AK. Mechanisms of airway protection after pharyngeal fluid infusion in healthy sleeping piglets. *J Appl Physiol* 78: 1942-1949, 1995.

Page M, and Jeffery HE. Airway protection in sleeping infants in response to pharyngeal fluid stimulation in the supine position. *Pediatr Res* 44: 691-698, 1998.

Page M, and Jeffery H. The role of gastro-oesophageal reflux in the aetiology of SIDS. *Early Hum Dev* 59: 127-149, 2000.

Perkett EA, and Vaughan RL. Evidence for a laryngeal chemoreflex in some human preterm infants. *Acta Paediatr Scand* 71: 969-972, 1982.

Pickens DL, Schefft G, and Thach BT. Prolonged apnea associated with upper airway protective reflexes in apnea of prematurity. *Am Rev Respir Dis* 137: 113-118, 1988.

Pickens DL, Schefft GL, and Thach BT. Pharygeal fluid clearance and aspiration preventive mechanisms in sleeping infants. *J Appl Physiol* 66: 1164-1171, 1989.

Reeh PW, and Kress M. Molecular physiology of proton transduction in nociceptors. *Curr Opin Pharmacol* 1: 45-51, 2001.

Reix P, Arsenault J, Dome V, Fortier PH, Lafond-Rouillard J, Moreau-Bussiere F, Dorion D, and Praud JP. Active glottal closure during central apneas limits oxygen desaturation in premature lambs. *J Appl Physiol* 94: 1949-1954, 2003.

Reix P, Fortier PH, Niyonsenga T, Arsenault J, Letourneau P, and Praud JP. Non nutritive swallowing and respiration coordination in full-term newborn lambs. *Respir Physiol Neurobiol* 134: 209-218, 2003.

Renolleau S, Letourneau P, Niyonsenga T, and Praud JP. Thyroarytenoid muscle electrical activity during spontaneous apneas in preterm lambs. *Am J Respir Crit Care Med* 159: 1396-1404, 1999.

Ross MG, and Nijland MJ. Development of ingestive behaviour. *Am J Physiol* 274: R879-893, 1998.

Roulier S, Arsenault J, Reix P, Dorion D, and Praud JP. Effects of C fiber blockage on cardiorespiratory responses to laryngeal stimulation in conscious lambs. *Respir Physiol Neurobiol* 136: 13-23, 2003.

Sladek M, Grogaard JB, Parker RA, and Sundell HW. Prolonged hypoxemia enhances and acute hypoxemia attenuates laryngeal reflex apnea in young lambs. *Pediatr Res* 34: 813-820, 1993.

Thach BT. Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to adult life. *Am J Med* 111: 69S-77S, 2001.

Van Der Velde L, Curran AK, Filiano JJ, Darnall RA, Bartlett D Jr, and Leiter JC. Prolongation of the laryngeal chemoreflex after inhibition of the rostral ventral medulla in piglets: a role in SIDS? *J Appl Physiol* 94: 1883-1895, 2003.

Wennergren G, Hertzberg T, Milerad J, et al. Hypoxia reinforces laryngeal reflex bradycardia in infants. *Acta Paediatr Scand* 78: 11-17, 1989.

Wetmore RF. Effects of acid on the larynx of the maturing rabbit and their possible significance to the sudden infant death syndrome. *Laryngoscope* 103: 1242-1254, 1993.