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Effects of reflux laryngitis on non-nutritive swallowing in newborn lambs

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ABSTRACT

Reflux laryngitis in infants may be involved not only in laryngeal disorders, but also in disorders of cardiorespiratory control through its impact on laryngeal function. Our objective was to study the effect of reflux laryngitis on non-nutritive swallowing (NNS) and NNS-breathing coordination. Two groups of six newborn lambs, randomized into laryngitis and control groups, were surgically instrumented for recording states of alertness, swallowing and cardiorespiratory variables without sedation. A mild to moderate reflux laryngitis was induced in lambs from the experimental group. A significant decrease in the number of NNS bursts and apneas was observed in the laryngitis group in active sleep ($p = 0.03$). In addition, lower heart and respiratory rates, as well as prolonged apnea duration ($p < 0.0001$) were observed. No physiologically significant alterations in NNS-breathing coordination were observed in the laryngitis group. We conclude that a mild to moderate reflux laryngitis alters NNS burst frequency and autonomous control of cardiac activity and respiration in lambs.

1. Introduction

Laryngopharyngeal reflux, which is very frequent in infants (Little et al., 1997; López-Alonso et al., 2006; Vandenplas et al., 1991), has been associated with several otolaryngologic manifestations including reflux laryngitis (Hawkshaw et al., 2013; Hom and Vaezi, 2013; Ulualp et al., 2007b). Previous reports have in turn ascribed cases of recurrent croup, laryngomalacia and subglottic stenosis in infants (Contencin and Narcy, 1992; Kwong et al., 2007; Rankin et al., 2013; Ulualp et al., 2007a,b) as well as apneas-bradycardias in newborns (Vermeylen et al., 2005) to reflux laryngitis. Such causative link may stem from alterations in pharyngolaryngeal reflexes secondary to reflux laryngitis, as reported in adult humans, suggesting a blunted protection against laryngeal penetration and tracheal aspiration (Ulualp et al., 1998). In addition, we previously showed an enhanced respiratory inhibition during laryngeal chemoreflexes in a lamb model of reflux laryngitis (Carreau et al., 2011). In some infants, alterations of these upper airway reflexes leading to laryngeal and/or tracheal chemoreflexes could contribute to cases of apnea of prematurity, apparent life-threatening events and sudden infant death syndrome (Leiter and Böhm, 2007; Praud, 2010; Thach, 2008).

Non-nutritive swallowing (NNS) occurrence and NNS-breathing coordination are of crucial importance in the infant in order to prevent laryngeal penetration. To the best of our knowledge, it is unknown whether reflux laryngitis alters NNS. The aim of the present study performed in our newborn ovine model was thus to test the hypothesis that the presence of reflux laryngitis decreases NNS frequency and alters NNS-breathing coordination.

2. Material and methods

2.1. Animals

Fourteen mixed-bred lambs were involved in the study. All lambs were born at term by spontaneous vaginal delivery at our local provider's farm and housed in our animal quarters from day one or two of life until the end of the experiments. The study protocol was approved by the Ethics Committee for Animal Care and Experimentation of our institution.

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2.2. Surgical instrumentation

Aseptic surgery was performed on the third day of life as previously described (St-Hilaire et al., 2010), under isoflurane anesthesia and after premedication with ketamine, atropine and morphine. Ketoprofen was added for analgesia and repeated if needed 12 h thereafter. Gentamicin and duplocilline were injected daily throughout the experimental days. All lambs were surgically instrumented for recording swallowing activity, states of alertness and cardiorespiratory variables. Custom-built needle-electrodes were inserted into both thyroarytenoid muscles (TA; a glottal adductor) through the lateral aspect of the thyroid cartilage for electrical activity (EMG) recording. Furthermore, needle-electrodes were inserted subcutaneously for recording electrocorticogram (ECoG), eye movements (EOG) and electrocardiogram (ECG). A catheter was introduced into the left carotid artery in order to monitor arterial blood gases throughout the experiment. Leads from all electrodes were subcutaneously tunneled to exit on the back of the lambs. Correct electrode positioning was systematically verified at necropsy. In addition, after identification of the thyroid gland, the esophagus was exposed caudally over 10 cm to visualize the right recurrent laryngeal nerve trajectory. Thereafter, a 20-Fr voice prosthesis (Blom-Singer ADVANTAGE Indwelling Voice Prosthesis, InHealth Technologies, Carpinteria, CA) was placed between the cervical esophagus and the skin to allow a one-way access to the esophagus. An 8 Fr Levine catheter was introduced through the prosthesis and pushed rostrally under direct laryngoscopic visualization, in such a way that the rostral extremity of the catheter was positioned 1–2 mm above the posterior rim of the larynx to allow laryngitis induction (see below). Finally, the caudal portion of the Levine catheter was placed around the lamb's neck under a bandage to provide easy access.

2.3. Reflux laryngitis induction

Lambs were randomly assigned to either the reflux laryngitis or the control group. As reported in a previous study performed in other lambs (Carreau et al., 2011), in the reflux laryngitis group, a 2 ml solution containing 300 U/ml of pepsin (Pepsin from porcine mucosa, P7012, Sigma–Aldrich Canada Ltd., Oakville, ON, Canada) and hydrochloric acid (HCl, pH 2.0) was instilled three times daily for six consecutive days via the esophageal catheter. A final instillation was performed on the last morning prior to the recording. This acidified pepsin solution, mimicking an acid gastric reflux, has been demonstrated to be pro-inflammatory to the laryngeal mucosa (Adhami et al., 2004; Carron et al., 2001; Koufman, 1991; Ludemann et al., 1998; Roh et al., 2006; Roh and Yoon, 2006). In addition, our solution was chosen to be representative of the gastric content of neonates, where pepsin concentration varies between 43 and 683 U/ml (Armand M, personal communication, Marseille, France). The same induction protocol was used for the control group using 2 ml of physiological saline (NaCl 0.9%).

2.4. Recording equipment

Instrumentation of the lamb was completed immediately before the recordings. Nasal flow was recorded with a double thermocouple wire (iron/constantan, type J; Omega Engineering, Stamford, CT) secured in an adapted dog muzzle. Respiratory inductance plethysmography was used to monitor respiratory thoraco-abdominal movements. A pulse oximeter sensor (Masimo Radical, Masimo®, Irvine, CA) was attached at the base of the tail for continuous monitoring of saturation and pulse wave. Finally, the Levine catheter was removed and replaced with a catheter-tipped pressure sensor (Microtip pressure transducer 9022K0902, Medtronic Minneapolis, MN, USA) inserted caudally into the esophagus to recognize whether laryngitis led to incomplete swallowing, i.e., with no esophageal deglutition. Data from prolonged recordings (with periods of wakefulness and sleep) in freely-moving non-sedated lambs was obtained using our custom-built radiotelemetry system (Samson et al., 2011). The telemetry transmitter was connected to the electrode leads and housed in the lamb's jacket. The raw EMG signals were rectified, integrated and averaged (moving time average = 100 ms). Polysomnographic signals were recorded on a PC, using the MP100A data acquisition system and AcqKnowledge software (version 3.7.3, Biopac Systems Inc., Santa Barbara, CA, USA). In addition, an observer was continuously present to note all events occurring during the recordings.

2.5. Design of the study

All lambs were housed with their mother in our animal quarters until the experiment. Surgical instrumentation was performed 24 hours after arrival in our animal quarters. Reflux laryngitis was induced for 6 consecutive days (postnatal days 3–8), beginning 24 hours after surgical instrumentation. Following the 6-day instillation of acidified pepsin (reflux laryngitis group) or NaCl 0.9% instillation (control group), polysomnographic recordings were performed in non-sedated lambs on postnatal day 9 between 6:00 AM and 12:00 PM. Lambs were placed in a Plexiglas chamber (1.2 m^3) in which temperature (24 °C) and humidity (70%) was maintained constant throughout the recording. An observer was also continuously present to note all events. Finally, following completion of the recording, euthanasia was performed by an intravenous injection of 100 mg/kg of pentobarbital sodium. Correct electrode and catheter positioning was systematically verified at necropsy.

2.6. Data analysis

2.6.1. Reflux laryngitis

Clinical follow-up was performed daily with lambs being weighed and examined in order to detect signs of laryngitis such as chronic cough, difficulties in feeding and raucous bleat (scored on a scale of 0–10). Correct positioning of the esophageal catheter was verified on the third or fourth day by X-ray imaging. Finally, histological assessment of laryngitis was performed using a scoring system initially developed for dogs, which we previously showed to be appropriate for lambs (Carreau et al., 2011; Duvareille et al., 2013; Koufman, 1991).

2.6.2. States of alertness

Standard electrophysiological (ECoG and EOG recordings) and behavioral criteria were used to define wakefulness (W), quiet sleep (QS), active sleep (AS) and arousals (Renolleau et al., 1999). Percentage of time spent in each state of alertness was calculated to determine any alterations of sleep architecture in reflux laryngitis lambs, as well as to document whether any modification in NNS frequency or NNS frequency coordination was state-dependent.

2.6.3. Cardiorespiratory variables

Heart and respiratory rates were calculated from the respiratory inductance plethysmography and the ECG signals during each period with a change in the state of alertness of more than 10 s; results were first averaged in each lamb, then in each group. Any presence of bradycardia (defined by a decrease >30% in heart rate) and desaturation (defined by a decrease in SpO_2 of at least 4% or <90%) was noted. Apneas were defined as two "missed" breaths, compared to the two preceding respiratory cycles. Apnea index (number of apneas per hour) and total duration of apneas were