Elsevier Editorial System(tm) for Respiratory Physiology & Neurobiology
Manuscript Draft

Manuscript Number: RESPNB2917R1

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Article Type: Short Communication

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Laryngeal narrowing during nasal ventilation does not originate from bronchopulmonary C-fibers

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Running title: C-fiber endings and laryngeal narrowing during nasal ventilation

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ABSTRACT

We previously showed that nasal pressure support ventilation (nPSV) can lead to active inspiratory laryngeal narrowing, which originates from the stimulation of bronchopulmonary receptors. Among the three major types of bronchopulmonary receptors, which are variably stimulated by lung distension, C-fiber endings are remarkable, given that their stimulation can also trigger laryngeal closure. Taking advantage of our lamb model with blocked C-fibers, we aimed to assess whether bronchopulmonary C-fiber endings are involved in the active inspiratory laryngeal narrowing during nPSV. Nine lambs were surgically instrumented to assess states of alertness, electrical activity of a glottal constrictor (EaTA), respiratory movements and arterial blood gases. Forty-eight hours later, two polysomnographic recordings were performed during nPSV 15/4 cmH_2O, before and after C-fiber blockade. During nPSV, blockade of C-fibers did not prevent inspiratory EaTA (present for 74 ± 41% of respiratory cycles *vs*. 64 ± 35%, p = 0.9). We conclude that active inspiratory laryngeal narrowing during nPSV does not originate from bronchopulmonary C-fiber endings.

KEYWORDS: Capsaicin, nasal pressure support ventilation, thyroarytenoid muscle, lamb

1. INTRODUCTION

Over the past few years, we have documented that nasal pressure support ventilation (nPSV) induces an active laryngeal narrowing during inspiration in non-sedated lambs (Moreau-Bussière et al., 2007; Roy et al., 2008). This inspiratory laryngeal narrowing can have deleterious consequences, including limitation of lung ventilation (Oppersma et al., 2013) as well as diversion of the insufflated gas into the esophagus. We further showed that this inspiratory laryngeal narrowing during nPSV was reflexively driven by the stimulation of unidentified bronchopulmonary receptors (Roy et al., 2008).

Vagal afferent messages from the lungs originate from three major types of bronchopulmonary receptors, namely slowly-adapting (SARs) and rapidly-adapting stretch receptors (RARs) and C-fiber endings (CFEs). The complex effects of lung inflation on these receptors have been summarized as follows (Coleridge JCG and Coleridge HM, 1984; Coleridge HM and Coleridge JCG, 2011). Slowly-adapting receptors are stimulated by moderate lung inflation and are involved in the inspiratory switch-off during normal breathing. RAR stimulation is usually secondary to rapid, large lung inflations, but can be seen even during normal breathing in some species. RAR stimulation provides a positive feed-back to inspiratory drive, which is deemed responsible for the occurrence of augmented breaths. Pulmonary CFEs are stimulated by large lung distension. i.e., at least 1 tidal volume above functional residual capacity. In addition, CFE stimulation can lead to active laryngeal closure, including in lambs (Diaz et al., 1999), which is of high relevance for our studies. From these

premises, the overall aim of our ongoing investigations is to uncover the bronchopulmonary receptor type(s) involved in the active, inspiratory laryngeal narrowing observed during nPSV. The specific aim of the present study was twofold: i) to take advantage of our unique newborn lamb model with blocked CFEs (Diaz et al., 1999) in order to assess whether CFEs are involved; ii) to perform exploratory experiments to test whether the few published tools used to modulate RAR or SAR function are operative in lambs.

2. MATERIALS AND METHODS

2.1- Animals

Nine male lambs aged 4 to 6 days and weighing 4.6 ± 0.8 kg were involved in the study. All lambs were housed in a Plexiglas chamber, where they were able to move freely and bottle-feed *ad libidum* on reconstituted ewe milk. The study was approved by the Ethics Committee for Animal Care and Experimentation of the Université de Sherbrooke (protocol # 037-10), in accordance with the Canadian Council on Animal Care guidelines.

2.2- Surgical Instrumentation and study design

Chronic instrumentation was performed under general anesthesia as previously detailed (Roy et al., 2008), in order to measure states of alertness, electrical activity of the thyroarytenoid muscle (EaTA, a glottal constrictor), tracheal $PetCO_2$, arterial blood gases and respiratory movements (inductance plethysmography). Two days later, in order to fulfill our first study aim, two polysomnographic recordings during nPSV 15/4 cmH_2O (Servo-i ventilator; inspiratory pressurization time = 0.12 s) were performed on two consecutive mornings, before (intact CFEs) and after CFE blockade, in non-sedated lambs. Neonatal injection of high doses of capsaicin is well known to induce both a selective degeneration and functional ablation of CFEs (Jancso et al., 1977), which we previously confirmed in newborn lambs (Diaz et al., 1999). Accordingly, CFEs were blocked on the first recording afternoon by a subcutaneous injection of 25 mg/kg of capsaicin (diluted in 10% Tween 80, 10% ethanol and 80% physiological saline) under a 30-min general anesthesia. The integrity of

bronchopulmonary CFEs was assessed by inducing pulmonary chemoreflexes by intravenous (IV) injections of 5 and 10 µg/kg capsaicin under intact CFE conditions, while effective CFE blockade was verified by IV injection of 50 µg/kg capsaicin (Diaz et al., 1999).

Following completion of the above experiments, selective blockade of RAR function was attempted in the last four non-sedated lambs. Increasing doses of dopamine (n = 2, IV infusion from 50 up to 200 µg/kg/min, after premedication with IV phentolamine + propanolol to prevent severe arterial hypertension) (Jackson and Simpson, 2000) or inhaled furosemide (n = 2, dose from 60 to 100 mg) (Sudo et al., 2000) were administered. RAR function was assessed *via* analysis of cardiorespiratory responses (number of coughs, apnea and bradycardia duration) to an intratracheal injection of 0.5 mL distilled H_2O. Finally, assessment of the Hering-Breuer inspiratory reflex (delayed onset of inspiration after nasal mask occlusion at end-inspiration) was also performed to confirm the absence of altered SAR function.

2.3- Data and statistical analyses

The number of respiratory cycles with inspiratory EaTA during a period of 60 s of quiet sleep was determined during nPSV 15/4 cmH_2O. The cardiorespiratory responses of the pulmonary chemoreflex, classically characterized by central apnea / bradycardia followed by tachypnea, were also analyzed.

The percentage of inspiratory EaTA as well as the cardiorespiratory responses following an IV injection of capsaicin were compared before and after CFE

blockade using the Wilcoxon signed-rank test. Differences were considered significant if $P < 0.05$. Data are expressed as mean ± SD.

3. RESULTS

3.1- Effectiveness of C-fiber blockade

In lambs with intact CFEs, 10 µg/kg capsaicin IV consistently induced pulmonary chemoreflexes with apnea + bradycardia lasting up to 11 s and preceding tachypnea (218 ± 53 respiratory cycles/min). This biphasic cardiorespiratory response was abolished in all lambs after CFE blockade, even following 50 µg/kg capsaicin IV (table 1).

3.2- Inspiratory laryngeal narrowing during nPSV

Eight of nine lambs with intact CFEs presented with inspiratory EaTA during nPSV 15/4 cmH_2O in quiet sleep. As illustrated in figure 1, CFE blockade did not prevent the occurrence of inspiratory EaTA (74 ± 41% vs. 64 ± 35% of respiratory cycles, $p = 0.5$). No differences were noted for $PaCO_2$ and PaO_2 (respectively $p = 0.4$ and $p = 0.6$) between before and after CFE blockade.

3.3- Attempts at selectively blocking RARs

Intravenous infusion of dopamine in two blocked-CFE lambs did not alter the cardiorespiratory responses to RAR stimulation, while the highest doses induced arterial hypertension. Similarly, inhaled furosemide did not alter the responses to RAR stimulation. As expected, no alteration of the Hering-Breuer inspiratory reflex was observed with either dopamine or furosemide.

4. DISCUSSION

The present study shows that the active inspiratory laryngeal narrowing observed during nasal pressure support ventilation in lambs does not originate from the stimulation of bronchopulmonary C-fiber endings.

4.1- Inspiratory laryngeal narrowing during nPSV

Over the past decade, we have shown in several studies that an active inspiratory narrowing develops against ventilator insufflations during nPSV (15/4 cmH_2O) in at least two thirds of full-term lambs in quiet sleep and quiet wakefulness (Moreau-Bussière et al., 2007; Roy et al., 2008). These results are in keeping with a recent review suggesting that this active inspiratory laryngeal narrowing can be responsible for significant patient-ventilator asynchrony, patient discomfort and non-invasive ventilation failure. As a result, further study of the mechanisms of this larynx-ventilator asynchrony has been advocated (Oppersma et al., 2013).

We previously documented that active inspiratory laryngeal narrowing during nPSV originates from bronchopulmonary receptors (Roy et al., 2008). The short burst of EaTA, disappearing well before the end of the inspiratory pressure plateau in nPSV (see figure 3 in Moreau-Bussière et al., 2007), does not suggest the involvement of SAR. While both RARs and CFEs are also stimulated by lung distension (Coleridge JCG and Coleridge HM, 1984; Coleridge HM and Coleridge JCG, 2011), our previous observation that stimulation of pulmonary CFEs in lambs triggers post-inspiratory EaTA (Diaz et al., 1999), together with the

availability of our robust lamb model with blocked CFEs, prompted us to assess the potential involvement of CFEs in inspiratory laryngeal narrowing during nPSV. The present demonstration that CEFs are not involved leads us now to suggest that this inspiratory laryngeal narrowing most likely originates from RAR stimulation.

4.2- Attempts at selectively blocking RARs

A systematic review of the literature revealed that IV dopamine (Jackson and Simpson, 2000) and inhaled furosemide (Sudo et al., 2000) can selectively block RARs, respectively in anesthetized adult dogs and rats. Unfortunately, we were unable to reproduce these results in lambs; such discrepancies may be related to differences in species, maturation and/or the use of anesthetics, all of which have previously been shown to affect bronchopulmonary receptors (Coleridge HM and Coleridge JCG, 2011).

A more comprehensive assessment of the three major types of bronchopulmonary receptors should include experimental modulation of SAR function. To our knowledge, the only report of SAR blockade in the literature involved the use of SO_2 inhalation. However, while the latter was successful in adult rabbits anesthetized with urethane, it was unsuccessful in newborn rabbits, in adult dogs and cats as well as in adult rabbits when using a different anesthetic agent (Mortola et al., 1984). Given these results and the current ban on urethane use due to its toxicity, no attempt was made to block SARs in lambs.

4.2- Conclusion

The present results showing that active inspiratory laryngeal narrowing during nasal pressure support ventilation does not originate from bronchopulmonary C-fiber endings suggest the likely involvement of bronchopulmonary rapidly adapting receptors. However, in the absence of available tools enabling the selective blockade of these receptors in lambs, such involvement remains to be demonstrated.

ACKNOWLEDGMENTS

This study was supported by the Canadian Institutes of Health Research. Jean-Paul Praud is the holder of the Canada Research Chair in Neonatal Respiratory Physiology and a member of the *Centre de recherche du Centre hospitalier universitaire de Sherbrooke*.

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FIGURE LEGENDS

Figure 1: A) Glottal constrictor activity (EaTA) during nasal support ventilation (nPSV) 15/4 cmH_2O before (intact C-fibers; left panel) and after C-fiber blockade (right panel). Abbreviations from top to bottom: EaTA, electrical activity of the thyroarytenoid muscle; ∫EaTA, moving time averaged EaTA; Pmask, mask pressure; Ptrach, tracheal pressure; Vlung, lung volume variations, given by the sum signal of the respiratory inductance plethysmography (inspiration upwards); i: inspiration; e: expiration. B) Percentage of respiratory cycles with inspiratory EaTA (% Inspir EaTA) before and after C-fiber blockade. Results reveal that blockade of bronchopulmonary C-fibers does not prevent inspiratory EaTA during nPSV 15/4 cmH_2O.

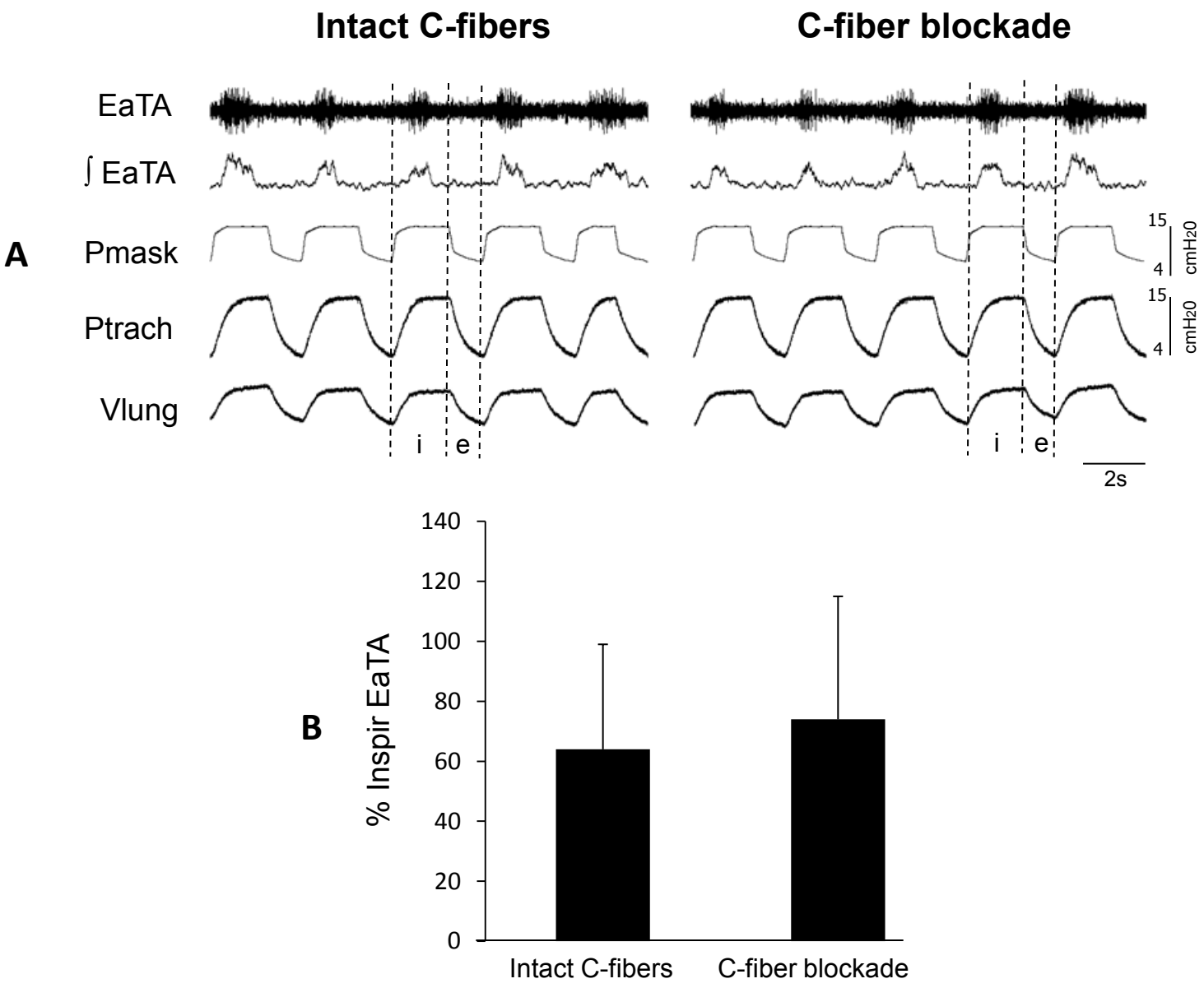
TABLES

Table 1: Cardiorespiratory responses following capsaicin IV injection before (intact C-fibers; 10 µg/kg) and after C-fiber blockade (50 µg/kg)

	Intact C-fibers	C-fiber blockade
Apnea duration, s	7 ± 4	1 ± 2
Bradycardia duration, s	6 ± 6	0.4 ± 0.9
Tachypnea, no of respiratory cycles/min	219 ± 53	0

Results are expressed as mean ± SD.

Figure 1


Intact C-fibers
C-fiber blockade
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EaTA
∫ EaTA
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Intact C-fibers
C-fiber blockade