Effects of C fiber blockade on cardiorespiratory responses to laryngeal stimulation in conscious lambs

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Abstract

The primary aim of the study was to explore cardiorespiratory reflexes originating from laryngeal C fiber endings in the neonatal period. Seventeen lambs were instrumented for recording glottal adductor and diaphragm EMG, heart rate, systemic arterial pressure and respiratory movements. C fiber blockade was induced in eight lambs by 30 mg/kg capsaicin, the remaining nine lambs serving as controls. Cardiorespiratory reflexes were induced in non-sedated lambs by flowing air, menthol or 13% CO2, or by injecting water or 50 mg capsaicin in the laryngeal inlet through an endoscope. Responses to all stimuli but capsaicin were similar between the two groups. While cardiorespiratory responses were induced by capsaicin in control lambs, the responses were significantly inhibited in lambs with C fiber blockade. We conclude that laryngeal C fiber endings are functional and responsible for laryngeal chemoreflexes in newborn lambs.

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Keywords: Airway, upper, laryngeal innervation; Development, cardio-respiratory reflexes, neonatal lamb; Mammals, lamb; Nerve, laryngeal C fibers; Pharmacological agents, capsaicin; Receptors, laryngeal C fibers; Reflex, laryngeal chemoreflexes

1. Introduction

The term ‘laryngeal chemoreflex’ has been repeatedly quoted to describe cardiorespiratory responses to stimulation of laryngeal mucosal receptors by liquids in immature newborn mammals. The laryngeal chemoreflexes include various combinations of the following responses: agitation or arousal, forceful active glottal closure (frequently laryngospasm), central or obstructive apnea, bradycardia and systemic arterial hypertension (Wetmore, 1993; Thach, 2001). They can be elicited by application of acid, water, or hyposmolar or hypochloric solutions into the laryngeal region. Clinical importance of the laryngeal chemoreflexes stems from the hypothesis that they are involved in apparent life threatening events in infants and some cases of sudden infant death.
syndrome (Wetmore, 1993; Page and Jeffery, 2000; Thach, 2001).

Several types of laryngeal sensory receptors have been described, responding to mechanical (pressure, flow/temperature, drive receptors) or chemical (ammonia, acid, CO₂, water, inhaled anesthetics) stimuli. A receptor can be responsive to several stimuli (Sant’Ambrogio et al., 1995; Takagi et al., 1995). Previous studies have suggested that C (unmyelinated) fibers are more abundant in sensory nerves in immature newborn mammals (including in the superior laryngeal nerve, SLN) than later in life (Chung et al., 1993), suggesting that C fibers may be specifically involved in the laryngeal chemoreflexes in newborn mammals. Some studies have assessed the involvement of SLN C fibers in cardiorespiratory reflexes elicited from laryngeal stimulation in adult, anesthetized mammals (Jammes et al., 1987; Palecek et al., 1990; Hishida et al., 1996; Naida et al., 1996; Lin et al., 2000; Mutoh et al., 2000a,b). However, to our knowledge, no studies have assessed the function of SLN C fibers in the neonatal period, though their relative abundance suggests that they have the potential to explain, at least in part, the laryngeal chemoreflexes specific to this period.

The aim of the study was to determine whether SLN C-fibers are functional in early life in lambs and whether they are responsible for the cardiorespiratory reflexes elicited by application of various stimuli onto the laryngeal region.

2. Methods

2.1. Animals

Seventeen mixed-breed full-term lambs were studied. The protocol of the study was approved by the University of Sherbrooke’s ethics committee for animal care and experimentation.

2.2. Surgical preparation

Surgery was performed within the first day of life under general anesthesia (Enflurane 2%+N₂O 30%+O₂ 68%), after premedication by atropine sulfate (100 µg/kg subcutaneously), and intramuscular ketamine 10 mg/kg (Ketaset, Ayerst, Montreal, PQ, Canada) and midazolam 100 µg/kg (Versed, Hoffman-La Roche, Mississauga, ON, Canada). Bipolar enameled chrome wire electrodes were inserted into the thyroarytenoid muscle (TA, a glottal adductor) and diaphragm (Di) muscles for recording electromyographic activity (EMG), as previously reported (Kianicka et al., 1994). Correct positioning of electrodes in laryngeal and diaphragmatic muscles was always verified at autopsy. An arterial catheter was also inserted into the brachial artery for recording systemic arterial pressure. Electrocardiographic (ECG) signals were recorded from two cup-electrodes placed 2 cm apart on the anterior thoracic wall. All leads were subcutaneously tunneled to exit on the lamb’s back.

At the end of the surgical procedure, eight of the lambs selected at random underwent C fiber blockade, using a previously published protocol (Diaz et al., 1999). A high dose of capsaicin (30 mg/kg) was injected subcutaneously under continuous anesthesia. The remaining nine lambs (control group) did not receive capsaicin.

All lambs returned to their mother after arousal from anesthesia. Post-operative care systematically included analgesia with one intramuscular injection of 0.005 mg/kg buprenorphine (Buprenex, Reckitt, Richmond, VA, USA) at the end of surgery. Moreover, daily antibiotic therapy with penicillin (Duplocillin LA, Intervet Canada, Whitby, ON, Canada) and gentamicin (Gentocin, Schering, Pointe-Claire, PQ, Canada) was given until completion of the experiments.

2.3. Measurement apparatus

Leads from the EMG and ECG electrodes and a nasal thermocouple were connected to a transmitter attached to the lamb’s back just prior to the experiment. The raw EMG and ECG signals and nasal flow were transmitted by radiotelemetry (Létourneau et al., 1999). The raw EMG signals were moving time averaged (100 ms) by the acquisition software (Acknowledge Version 3.2, Biopac System Inc., Santa Barbara, CA, USA). Thoracic and abdominal volume variations were
qualitatively assessed using respiratory inductance plethysmography (Respitrace, NIMS, Miami Beach, FL, 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).

Raw and integrated EMG signals, thoracic and abdominal volume variations and their sum, nasal flow, ECG signal, and systemic arterial pressure were continuously recorded using an Apple Macintosh microcomputer and the Biopac software. Collected data were stored on compact disk for further analysis. Finally, the working channel of an endoscope (3.5 mm diameter, VFS-2A, Machida, Norwood, NJ, USA) was used to deliver gases and liquids under direct vision into the laryngeal inlet.

2.4. Design of the study

The lambs were comfortably positioned in a sling with loose restraints. Ambient temperature was kept between 20 and 22 °C. Experiments were performed in non-sedated, conscious lambs during quiet wakefulness and began with a 3-min baseline recording (Fig. 1). The endoscope lubricated with jelly was then gently introduced through one nare until the distal end was positioned just over the laryngeal inlet, aiming at directing the outlet of the working channel onto the interarytenoid space. Gas stimuli included 5 L/min humidified air at 37 °C, and 5 L/min humidified air at 37 °C with L-menthol (Sant’Ambrogio et al., 1992) during 3 s. A 60 ml bottle containing 100 mg L-menthol was placed in series with the airflow delivery system. Distilled water (1 ml) was given as a bolus injection. Stimuli were applied in random order through the working channel of the endoscope. All above stimuli were repeated once, with at least a 1 min interval between 2 stimuli. Finally, the experiment was ended by giving a bolus injection of 1 ml of 50 μg capsaicin (Sigma Chemical, St. Louis, MO, USA) in Tween 80+10% ethanol. Capsaicin injection was not repeated. The same investigator (J-P P) was assigned to manipulate the endoscope in all lambs. The events noted on computer for each challenge included swallowing, agitation, cough and apnea >3 s.

Effectiveness of C fiber blockade was verified at the end of the experiments by recording the pulmonary chemoreflex induced by intravenous capsaicin at increasing doses (5–50 μg/kg) in 4 control and all 8 C fiber-blocked lambs. In accordance with previous studies in our laboratory, the dose judged as eliciting a significant pulmonary chemoreflex was that which elicited an initial apnea >10 s or a secondary increase in breathing frequency higher than three times the baseline value (Diaz et al., 1999). The lambs were systematically euthanized at the end of the experiments by one IV injection of pentobarbital (50 mg/kg).

2.5. Data analysis

The first objective of the study was to assess whether CO₂ and L-menthol elicited specific cardiorespiratory responses, i.e. significantly greater than the responses to air, in awake, non-sedated control lambs. Simultaneously, the potency of the laryngeal responses to CO₂, L-menthol and water was compared. The second, main objective of the study was to assess whether laryngeal C fibers were involved in the cardiorespiratory responses elicited by various laryngeal stimuli. This was conducted in two steps. The first step was to test the hypothesis that laryngeal C fibers are functional in non-sedated, awake lambs. This was assessed by comparing the cardiorespiratory responses to
laryngeal instillation of capsaicin in control lambs and in C fiber-blocked lambs. The second step was to study whether the significant cardiorespiratory responses obtained with the stimuli studied in the first part of the study were significantly inhibited by C fiber-blockade. This was assessed by comparing the responses to the stimuli obtained in control vs. C fiber-blocked lambs.

Analysis of the cardiorespiratory responses to each laryngeal stimulus was performed in several ways, as follows. First, the time interval between onset of cardiorespiratory responses and resumption of three normal breaths, the laryngeal reflex duration, was manually measured on all recordings. Secondly, we counted the number of several events occurring within the 30 s-interval following laryngeal stimulation, including apneas > 3 s, swallowing (from TA EMG, available in 7 control and 8 C fiber-blocked lambs), coughing and agitation (both noted by one observer during the recording). Total summed duration of apneas > 3 s and of agitation periods were also calculated for each stimulus within the 30 s-interval following laryngeal stimulation. Thirdly, we assessed whether laryngeal stimulation induced bradycardia and/or systemic arterial hypertension, as part of the laryngeal chemoreflexes elicited by liquids (Wetmore, 1993). The percentage decrease in heart rate \( \% \text{decHR} = (\text{HR}_{\text{BL}} - \text{HR}_{\text{min}}) \times 100/\text{HR}_{\text{BL}} \) was calculated, with \( \text{HR}_{\text{BL}} \) being the baseline HR value (averaged on a 10 s-period, within 20 s before challenge) and \( \text{HR}_{\text{min}} \) the minimal HR value observed within 30 s after challenge. Similarly, the percentage of increase in mean arterial pressure \( \% \text{incMAP} = (\text{MAP}_{\text{max}} - \text{MAP}_{\text{BL}}) \times 100/\text{MAP}_{\text{BL}} \) was calculated, with \( \text{MAP}_{\text{BL}} \) being the baseline mean arterial pressure (MAP) value (averaged on a 10 s-period, within 20 s before challenge) and \( \text{MAP}_{\text{max}} \) the maximal MAP value observed within 30 s after challenge.

2.6. Statistical analysis

Measurements were averaged first in each lamb, then for the group as a whole for each test. Group means were reported as means ± standard deviation. Differences between group mean values in control vs. C fiber blocked lambs were assessed using the Mann–Whitney U-test for unpaired comparisons. Differences of responses between the various laryngeal stimuli were assessed by one factor ANOVA, completed by post-hoc test comparisons (PLSD Fisher exact test) when applicable (Statview Abacus Concepts, Berkeley, CA, USA). A \( P \leq 0.05 \) was taken as statistically significant.

3. Results

No significant differences were found between control and C fiber-blocked lambs for age (respectively \( 2.6 \pm 0.5 \) vs. \( 3.3 \pm 1.3 \) days, \( P = 0.22 \)) or weight (respectively \( 5.1 \pm 1.0 \) vs. \( 4.3 \pm 0.6 \) kg, \( P = 0.06 \)) on the experimental day. Moreover, no significant differences were found between control and C fiber-blocked lambs for respiratory rate (respectively \( 43 \pm 10 \) vs. \( 37.5 \pm 10 \) breaths/min, \( P = 0.34 \)), HR (respectively \( 228 \pm 26 \) vs. \( 194 \pm 36 \) beats/min, \( P = 0.77 \)) and MAP (respectively \( 80 \pm 14 \) mmHg vs. \( 77 \pm 14 \) mmHg, \( P = 0.64 \)).

C fiber blockade was shown to be highly effective in all eight lambs. Indeed, while a pulmonary chemoreflex could be elicited with IV injection of 5 μg/kg capsaicin in control lambs, no significant pulmonary chemoreflex could be elicited with up to 50 μg/kg capsaicin in any of the 8 C fiber blocked lambs. However, as a weak response was elicited in all eight lambs with the highest doses of capsaicin, C fibers were not totally blocked. Thus, the appellation ‘C fiber blocked lambs’ throughout the text means ‘partially C fiber blocked lambs’.

3.1. Cardiorespiratory responses to laryngeal stimulation in awake, non-sedated, control lambs

Results are reported in Table 1. A flow of 5 L/min air into the interarytenoid area elicited mild responses in control lambs, including transient agitation in half of the tests (mean duration = 1.7 s ± 2) and swallowing movements (mean number = 2.9 ± 2.5 per test). Coughing, apnea, decrease in HR or increase in MAP were virtually absent. Total duration of the laryngeal reflexes was 4.6 ± 4.3 s. The cardiorespiratory responses to
Table 1
Cardiorespiratory responses to various laryngeal stimuli in newborn lambs

<table>
<thead>
<tr>
<th></th>
<th>Air C</th>
<th>B</th>
<th>t-Menthol C</th>
<th>B</th>
<th>CO₂ C</th>
<th>B</th>
<th>H₂O C</th>
<th>B</th>
<th>Capsaicin C</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apnea index</td>
<td>0.1±0.2</td>
<td>0 ±0</td>
<td>0.1±0.2</td>
<td>0 ±0</td>
<td>0 ±0</td>
<td>0.1±0.3</td>
<td>0.3±0.3</td>
<td>0.3±0.3</td>
<td>–</td>
<td>0.4±0.7</td>
</tr>
<tr>
<td>Swallowing index</td>
<td>2.9±2.5</td>
<td>1.6±1.4</td>
<td>2.6±2</td>
<td>2.9±1.8</td>
<td>1.2±1.2</td>
<td>1.1±0.9</td>
<td>9.9±9.2*</td>
<td>13.1±5.9</td>
<td>14.9±8.4</td>
<td>8.3±5.6</td>
</tr>
<tr>
<td>Coughing index</td>
<td>0±0</td>
<td>0±0</td>
<td>0.1±0.2</td>
<td>0±0</td>
<td>0±0.4</td>
<td>0±0</td>
<td>0.8±1**</td>
<td>0.3±0.4</td>
<td>1.7±1.8</td>
<td>0.6±0.7</td>
</tr>
<tr>
<td>Apnea duration (s)</td>
<td>0.2±0.7</td>
<td>0±0</td>
<td>0.6±1.2</td>
<td>0±0</td>
<td>0±0.5</td>
<td>1.2±1.5*</td>
<td>1.6±2.9</td>
<td>–</td>
<td>2.7±4.9</td>
<td>–</td>
</tr>
<tr>
<td>Agitation duration (s)</td>
<td>1.7±2</td>
<td>0±0</td>
<td>2±3</td>
<td>0.7±1.3</td>
<td>0.9±1.7</td>
<td>0±0</td>
<td>5.8±7.9</td>
<td>5.1±4.5</td>
<td>21.6±3.8***</td>
<td>4.6±6.1****</td>
</tr>
<tr>
<td>Reflex duration (s)</td>
<td>4.6±4.3</td>
<td>3.9±2.7</td>
<td>7±4.9</td>
<td>6±2.7</td>
<td>3.2±3.2</td>
<td>2.7±2.9</td>
<td>13.5±7.3*</td>
<td>16.4±6.1</td>
<td>33.4±13.8***</td>
<td>16.6±9.9****</td>
</tr>
<tr>
<td>%decHR</td>
<td>8±15</td>
<td>8±10</td>
<td>10±17</td>
<td>7±8.5</td>
<td>8±8</td>
<td>5±10</td>
<td>27±13*</td>
<td>22±11</td>
<td>26±14*</td>
<td>15.7±11</td>
</tr>
<tr>
<td>%incMAP</td>
<td>7±11</td>
<td>11±1</td>
<td>5±10</td>
<td>13±6.8</td>
<td>6±7</td>
<td>10±5</td>
<td>16±14</td>
<td>30±24</td>
<td>41±11***</td>
<td>17±12****</td>
</tr>
</tbody>
</table>

C: control lambs. B: C fiber-blocked lambs. Apnea, swallowing, coughing index: number of events within 30 s following the stimulus. Apnea and agitation duration: summed duration of the time spent in apneas or agitation within 30 s following the stimulus. Reflex duration: time duration between the stimulus and resumption of three normal breaths. %decHR: percentage of decrease in heart rate (see text). %incMAP: percentage of increase in systemic arterial pressure (see text). All results are given as means ± standard deviation.

* P < 0.05 vs. air, t-menthol and CO₂.
** P = 0.05 vs. air.
*** P < 0.05 vs. air, t-menthol, CO₂ and water.
**** P < 0.05 vs. control lambs.
of the laryngeal reflexes \( (P \leq 0.002 \text{ vs. air, L-menthol and CO}_2) \). Coughing was also significantly more marked after water than after air stimulation \( (P = 0.05) \). Agitation and \%incMAP were not significantly greater after water stimulation than after air stimulation \( (P > 0.05, \text{ water vs. air, L-menthol and CO}_2) \).

### 3.2. Presence of functional laryngeal C fibers

Results on the function of laryngeal C fibers are also reported in Table 1. Injection of capsaicin (Fig. 5) elicited potent cardiorespiratory responses beginning immediately after stimulation, including marked agitation, repeated swallowing movements and coughing in all tests. Agitation often precluded recognition of apneas. The \%incMAP, the summed duration of agitation periods and the total duration of the laryngeal reflexes were significantly higher than that observed in response to the other laryngeal stimuli, including water \( (P < 0.001) \). The \%decHR was significantly greater than that observed in response to air, L-menthol and CO\(_2\) \( (P < 0.03) \), but identical to that observed with water stimulation \( (P = 0.89) \).

No significant differences were found between control and C fiber-blocked lambs for all parameters analyzed with respect to swallowing,
coughing, apnea, agitation, duration of the laryngeal reflexes, %decHR and %incMAP observed in response to L-menthol, CO₂ and water ($P$ between 0.27 and 1). Conversely, several responses to capsaicin were significantly blunted in C fiber blocked lambs as compared to control lambs, including agitation ($P < 0.002$), duration of the laryngeal reflexes ($P < 0.03$), and %incMAP ($P = 0.02$). Blunting of the other responses to capsaicin, including coughing ($P = 0.20$), swallowing ($P = 0.12$) and %decHR ($P = 0.18$) was not significant (Fig. 6).

4. Discussion

The present study is one of the few to report the cardiorespiratory responses to various laryngeal stimuli in an awake, non-sedated newborn mammal. Overall, aside from capsaicin, water was the only tested stimulus capable of eliciting significant cardiorespiratory reflexes. More importantly, the present study shows for the first time that cardiorespiratory reflexes can be elicited in a newborn mammal by stimulation of laryngeal C fibers. In addition, our findings suggest that the cardiorespiratory reflexes induced by laryngeal application of distilled water do not originate from C fibers.

These findings on laryngeal C fibers in awake, non-sedated lambs, are in agreement with results previously reported in anesthetized adult mammals. The importance of these unique findings is related to the potency of the laryngeal chemoreflexes in newborn mammals. These reflexes are known to be frequently involved in infants with apparent life-threatening events and may be responsible for some cases of sudden infant death syndrome.

4.1. Cardiorespiratory responses to laryngeal stimulation with gases or water in the awake, non-sedated lamb

4.1.1. Responses to CO₂

High concentrations of CO₂ ($> 5\%$) flowing through the larynx have been shown to decrease ventilation in adult, anesthetized or decerebrate mammals (Anderson et al., 1990; Nolan et al., 1990; Bartlett et al., 1992; Sant’Ambrogio et al., 1995; Bradford et al., 1998). While CO₂-sensitive laryngeal receptor endings are mostly irritant-type receptors in adult life (Anderson et al., 1990; Sant’Ambrogio et al., 1995), some are stimulated by negative pressure (Anderson et al., 1990; Bradford et al., 1998) or cooling (Bradford et al., 1998). The effects of CO₂ on laryngeal receptors are largely unknown in the neonatal period. A
decrease in minute-ventilation with apnea in response to CO₂ (>6%) flowing through whole upper airways has been reported in human pre-term infants (Alvaro et al., 1992). However, a flow of 10% CO₂ through the whole upper airways has been reported to increase minute-ventilation and upper airway resistance in anesthetized, tracheotomized infant guinea-pigs. These reflexes persisted following SLN section, but were abolished following topical anesthesia of the whole upper airways, suggesting that upper airway receptors involved were not (or not only) at the laryngeal level (Curran et al., 1996, 2000). To our knowledge, the present study is the first to specifically assess the responses to CO₂ at the laryngeal level in the neonatal period, and the first in sheep. Whether the absence of response to laryngeal stimulation by CO₂ observed in the present study is representative of the neonatal period, species-related, or due to the experimental design, including a too short time of laryngeal exposition to CO₂, remains unanswered.

4.1.2. Responses to L-menthol

The most consistent effect of cooling (decrease of 5–10 °C) and L-menthol, a specific stimulant of cold receptors, is to depress ventilation, particularly in newborn animals (Al-Shway and Mortola, 1982; Fisher et al., 1985; Mathew et al., 1986; Sant’Ambrogio et al., 1992; Anderson and Fisher, 1994; Curran et al., 1995, 1998). Results in anesthetized puppies suggest however that the ventilatory inhibition could be mainly due to stimulation of nasal cold receptors (Sant’Ambrogio et al., 1992). The absence of cardiorespiratory responses attributable to discrete stimulation of the larynx by L-menthol observed in the present experiments provides some support to this suggestion. Alternatively, the absence of recognizable responses in awake lambs may be due to species differences or experimental design, including a too short time of laryngeal exposition to menthol.

4.1.3. Responses to water

Distilled water is one of the most potent stimuli of laryngeal receptors, eliciting a response in over 80% of chemosensitive primary afferent fibers and nucleus tractus solitarius neurons in the adult cat (Takagi et al., 1995). Since the first observation by Johnson et al. (1972) in anesthetized lambs, numerous studies have shown that the cardiorespiratory responses to water are especially marked in newborn mammals (Wetmore, 1993; Thach, 2001). Boggs and Bartlett (1982) have shown that low chloride concentration was responsible for the response to water. Distilled water frequently elicits marked laryngeal chemoreflexes (laryngospasm, apnea, bradycardia and systemic hypertension) in anesthetized newborn mammals, at times leading to death (Downing and Lee, 1975). To our knowledge, four studies have been conducted in non-sedated newborns, including two in human pre-term newborns (Davies et al., 1988; Page and Jeffery, 1998), one in piglets (Page et al., 1995), and one in preterm lambs (Marchal et al., 1982). Laryngeal chemoreflexes, including apnea, hypertension and bradycardia, which were more marked in sleep than during wakefulness, were reported in two of the studies (Marchal et al., 1982; Davies et al., 1988). Our findings confirm that laryngeal instillation of water leads to depression of ventilation, coughing, swallowing and bradycardia in awake, non-sedated full-term lambs. These responses contrast with the very mild and seemingly not specific responses obtained with air, CO₂ and L-menthol in the present study.

4.2. Laryngeal C fibers

Stimulation of laryngeal C fiber endings in anesthetized adult mammals leads to various cardiorespiratory responses, including prominent apneas. Laryngeal C fiber endings are sensitive to various chemical stimuli such as capsaicin in dogs, rats and guinea-pigs (Forsberg et al., 1988; Palecek et al., 1990; Mutoh et al., 2000a), phenyl diguanide in cats (Boushey et al., 1972; Jammes et al., 1987), citric acid in guinea pigs (Forsberg et al., 1988), ammonia in rats (Naida et al., 1996), volatile anesthetics in dogs (Mutoh et al., 1998), and to cooling and mechanical stimulation in cats (Jammes et al., 1987). By contrast, laryngeal C fiber endings are insensitive to water in dogs (Mutoh et al., 2000b) and guinea-pigs (Forsberg et al., 1988), and are not involved in the apneic response to wood smoke in rats (Lin et al., 2000).
Stimulation by capsaicin has traditionally been used to distinguish C fibers from myelinated fibers (Coleridge and Coleridge, 1984), and C fiber endings are often referred to as to capsaicin-sensitive receptors. While it is now known that capsaicin is a selective stimulant for both C fiber endings and some A-δ fiber endings (Holzer, 1991; Mohammed et al., 1993; Riccio et al., 1996), the use of capsaicin is still considered to be an advantageous method for identifying C fiber endings, including in the laryngeal mucosa (Mutoh et al., 1998). Moreover, the involvement of laryngeal C fibers in response to a particular stimulus can be recognized after blockade of C fiber function, either by systemic administration of capsaicin or ruthenium red (Naida et al., 1996; Lin et al., 2000), or by perineural application of capsaicin on SLN (Naida et al., 1996; Lin et al., 2000; Mutoh et al., 2000a,b).

While in adult mammals the reported proportion of C fibers in the internal branch of the SLN ranges from 11% in cats (Miller and Loizzi, 1974), to near 50% in dogs (Chung et al., 1993), rats and guinea pigs (Hishida et al., 1997), C fibers constitute 81% of the SLN fibers in the newborn dog (Chung et al., 1993). This may suggest a more important role for reflexes elicited from laryngeal C fiber endings in the neonatal period. However, it is not known whether laryngeal C fibers have a higher threshold for elicitation of reflexes in the newborn mammal, as generally acknowledged for pulmonary C fibers and the pulmonary chemoreflex (Mortola, 2001). The present study was not designed to test whether stimulation of laryngeal C fibers is capable of eliciting more potent cardiorespiratory reflexes in lambs than in adult sheep. However, to our knowledge, it provides the first demonstration that stimulation of laryngeal C fibers elicits cardiorespiratory reflexes in the sheep, and, above all, that laryngeal C fibers are functional in the neonatal period.

4.3. Laryngeal C fibers and responses to water

Our findings suggest that the cardiorespiratory responses to laryngeal stimulation by water are not conveyed by SLN C fibers in lambs, because it is not significantly inhibited by C fiber blockade. This is in agreement with previous studies in adult dogs, following selective blockade of laryngeal C fibers by topical laryngeal capsaicin (Mutoh et al., 1997) or capsaicin perineural treatment (Mutoh et al., 2000b). However, if the responses to water application are mediated by several types of sensory endings, the involvement of C fiber blockade may have been obscured. The absence of a decrease in swallowing movements in response to water, following C fiber blockade, in the present study, may seem at variance with previous findings in guinea pigs (Jin et al., 1994). However, we have previously observed an increase in spontaneous non-nutritive swallowing in C fiber blocked lambs (unpublished results). Whether these discrepancies are due to differences in animal species or age is not known. Our study is the first to show that laryngeal C fibers are not sensitive to water in the neonatal period, when laryngeal chemoreflexes in response to water are maximal and C fibers are predominant within the SLN. Water presumably specifically stimulates myelinated receptors such as rapidly adapting receptors and respiration-modulated pressure or drive receptors (Sant’Ambrogio et al., 1995). Similarly, laryngeal responses to wood smoke were shown not to originate from capsaicin-sensitive receptors in anaesthetized rats (Lin et al., 2000). Conversely, previous data suggest that C fiber endings are involved in the responses to acid (Forsberg et al., 1988), which is independent from the response to low chloride content (Wetmore, 1993; Thach, 2001). This may be especially relevant in the neonatal period, when C fibers are functional (present findings) and more numerous (Chung et al., 1993), and where apparent life-threatening events are frequently observed in response to acid gastro-esophageal reflux.

5. Conclusion

Our findings show that laryngeal C fibers are functional and responsible for eliciting laryngeal chemoreflexes in awake, non-sedated lambs. Following this first demonstration that laryngeal C fibers are functional in the neonatal period, further studies will be needed to elucidate their role in the newborn. This includes C-fiber response to acid.
stimulation, which is highly relevant in clinical situations in infants with pathological acid gastro-esophageal reflux.

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