Effects of capsaicin pretreatment on expiratory laryngeal closure during pulmonary edema in lambs

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Diaz, Véronique, Dominique Dorion, Sylvain Renolleau, Patrick Létourneau, Irenej Kianicka, and Jean-Paul Praud. Effects of capsaicin pretreatment on expiratory laryngeal closure during pulmonary edema in lambs. J. Appl. Physiol. 86(5): 1570–1577, 1999.—The present study, performed in nonsedated, conscious lambs, consisted of two parts. In the first part, we 1) examined for the first time whether a respiratory response to pulmonary C-fiber stimulation could be elicited in nonsedated newborns and 2) determined whether this response could be abolished by capsaicin pretreatment. Then, by using capsaicin-desensitized lambs, we studied whether pulmonary C fibers were involved in the sustained, active expiratory upper airway closure previously observed during pulmonary edema. Airflow and thyroarytenoid and inferior pharyngeal constrictor muscle electromyographic activities were recorded. In the first set of experiments, a 5–10 µg/kg capsaicin bolus intravenous injection in seven intact lambs consistently led to a typical pulmonary chemoreflex, showing that C fibers are functionally mature in newborn lambs. In the second series of experiments, eight lambs pretreated with 25–50 mg/kg subcutaneous capsaicin did not exhibit any respiratory response to 10–50 µg/kg intravenous capsaicin injection, implicating C fibers in the response. Finally, in the above capsaicin-desensitized lambs, we observed that halothane-induced high-permeability pulmonary edema did not cause the typical response of sustained expiratory upper airway closure seen in the intact lamb. We conclude that functionally mature C fibers are present and responsible for a pulmonary chemoreflex in response to capsaicin intravenous injection in nonsedated lambs. Capsaicin pretreatment abolishes this reflex. Furthermore, the sustained expiratory upper airway closure observed during halothane-induced pulmonary edema in intact nonsedated lambs appears to be related to a reflex involving stimulation of pulmonary C fibers.

WE HAVE SHOWN that an active expiratory upper airway closure is consistently observed in newborn lambs with induced pulmonary edema (10). Furthermore, our companion paper suggests that this expiratory upper airway closure is related to vagal afferents originating from bronchopulmonary receptors since vagotomy abolishes the response (10). Various bronchopulmonary receptors, including slowly adapting receptors (24), rapidly adapting receptors (20–22), and C-fiber endings (1, 21, 23), are known to be activated during pulmonary edema. We report in a companion paper that a decrease in lung volume induced by injection of saline in the pleural space does not trigger expiratory upper airway closure in lambs (10). This led us to hypothesize that pulmonary C fibers, which are stimulated by pulmonary congestion (9), might play an important role in triggering expiratory upper airway closure during pulmonary edema in newborn lambs. This hypothesis is further complicated by questioning about C-fiber function in the neonatal period. Whereas early attempts to identify the existence of functionally mature pulmonary C fibers in newborn mammals were inconclusive (12, 14, 17), recent data in anesthetized newborn dogs indicated that reflex responses can be elicited by presumptive C-fiber stimulation with capsaicin (2). It is, however, still to be proven whether pulmonary C fibers are functional in nonsedated, conscious newborns, in our case the lamb.

The present study can be subdivided in two equally important parts. Specific aims of the first part were 1) to test for the presence of a pulmonary chemoreflex in response to an intravenous bolus injection of capsaicin in nonsedated lambs and 2) to test whether capsaicin desensitization procedures previously described in rodent species have similar actions on the reflex responses of the newborn lamb. In the second part, our aim was to establish whether capsaicin-desensitization inhibits the sustained expiratory upper airway closure observed during pulmonary edema in intact lambs.

MATERIALS AND METHODS

Animals

Fifteen lambs aged 4–19 days and weighing 4–6.3 kg were involved in the study. Lambs were divided into two groups: 1) seven intact lambs aged 4–19 days and weighing 5.1–6.3 kg and 2) eight lambs aged 4–11 days and weighing 3.2–5.6 kg that had received very high doses (25–50 mg/kg) of subcutaneous capsaicin in the first 3 days of life. These lambs were referred hereafter as "capsaicin-desensitized lambs." All lambs were born at term by spontaneous vaginal delivery and housed with their mothers in our animal quarters for a few days before the experiments. The protocol of the study was approved by our institution's ethics committee for animal research.

Surgical Preparation

Aseptic surgery and subcutaneous injections of capsaicin were performed while the lambs were under general anesthesia (2% halothane-40% N2O-58% O2) after premedication by...
an intramuscular injection of ketamine (10 mg/kg) and acepromazine (0.1 mg/kg) and a subcutaneous injection of atropine (0.2 mg/kg).

Muscle electrode placement. Intramuscular bipolar electrodes (enameled chrome wire, 0.7-mm diameter; Chromel, GTSM, Castelnaudary, France) were inserted under direct vision into both thyroarytenoid muscle (TA) and inferior pharyngeal constrictor muscle (IPC) in all lambs. Details with regard to electrode insertion have been described previously (11, 18). The leads were subcutaneously tunneled to exit on the back of the animals. Electrode placement was confirmed by systematic autopsy after completion of the experiment in all lambs. A catheter was also inserted into the brachial artery in capsaicin-desensitized lambs (7 lambs).

Systemic injection of high doses of capsaicin. Subcutaneous injections of 25–50 mg/kg of capsaicin (Sigma Chemical, St. Louis, MO) were performed at the end of the surgical procedure in eight lambs. To prevent bronchospasm and pain, each injection was preceded by a subcutaneous injection of 25–50 mg/kg of capsaicin (Sigma Chemical, St. Louis, MO). As an additional precaution, each lamb received intravenous atropine (0.4 mg·kg⁻¹·h⁻¹), continuous intravenous salbutamol (0.8 µg·kg⁻¹·h⁻¹), subcutaneous epinephrine (10 µg·kg⁻¹·h⁻¹), intravenous bunoperphine (5 µg·kg⁻¹·h⁻¹), and intravenous ketamine (10 mg·kg⁻¹·h⁻¹). Heart rate and transcutaneous arterial oxygen saturation (SaO₂) were monitored throughout capsaicin desensitization. The first five lambs received 50 mg/kg capsaicin diluted in DMSO and divided into four increasing doses (3.5, 7, 20, and 20 mg/kg); volume of each injection was 10 mL. One lamb died during surgery, and another died within 2 days after capsaicin injection. Because two of the first five lambs did not survive the capsaicin-DMSO injections, we altered the injections in the remaining three lambs. We therefore diluted capsaicin in Tween 20 instead of DMSO and injected one-half the dose of capsaicin (25 mg/kg) divided into three subcutaneous injections (5, 10, and 10 mg/kg) for the last three lambs. After each injection (volume = 10 mL), there was an increase in heart rate (up to 200 beats/min); the following injection was performed when heart rate had regained baseline values (within 30 min after injection). There was no decrease in SaO₂ after capsaicin injections.

Postoperative period. An intramuscular injection of bunoperphine (5 µg/kg) was systematically given at the end of surgery. Furthermore, an intramuscular injection of penicillin and gentamicin was given daily until the experimental day.

Measurement Apparatus

The measurement apparatus used in the present experiments has been previously described (18). Briefly, a face mask, specifically designed for each lamb, was attached to a size-0 pneumotachograph (model 2107B plus model 8815A respiratory integrator, Hewlett-Packard, Waltham, MA). The TA and the IPC electromyographic (EMG) activity signals were amplified and band-pass filtered at 30–1,000 Hz (model P511 alternating-current preamplifier and 7DA direct-current driver amplifier, Grass, Quincy, MA) before undergoing rectification and 100-ms moving time averaging (Dept. of Electronics, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, PQ). The integrator used had parallel outputs to a chart recorder and a computer. A 10-channel polygraph (model 7D, Grass) recorded instantaneous airflow, tidal volume (VT), and the raw and integrated EMG signals of both muscles. An IBM-compatible microcomputer analyzed airflow and integrated EMG signals at a 40-Hz sampling rate. Collected data were stored on disk for further analysis.

Design of the Study

All experiments were performed in nonsedated conscious lambs, at least 2 days after surgery and high-dose capsaicin injections. Each lamb was comfortably positioned in a sling, and a face mask was connected to a pneumotachograph carefully applied on its snout. A venous catheter was inserted into the superior vena cava through the superficial jugular vein under local anesthesia (2% lidocaine). Each experimental procedure began with a 5-min baseline recording. Ambient temperature was kept between 20 and 22°C and humidity between 50 and 70% throughout the experiment. Lambs were systematically killed after completion of experiments by intravenous injection of 50 mg/kg pentobarbital sodium.

Intravenous bolus injection of capsaicin. After 3 min of room-air baseline recording, lambs were given an intravenous bolus injection of capsaicin at increasing doses (5, 10, 25, and 50 µg/kg) over 2–6 s until the expected classic pulmonary chemoreflex was observed, i.e., a central apnea followed by rapid shallow breathing. This experiment was first conducted in intact lambs to verify whether pulmonary C fibers could be reflexly activated by capsaicin injection in the neonatal period. The same procedure was then repeated in capsaicin-desensitized lambs to examine whether the pulmonary chemoreflex could be permanently inhibited.

High-permeability pulmonary edema in capsaicin-desensitized lambs. To study the role of pulmonary C fibers on expiratory upper airway closure during pulmonary edema, respiratory parameters with TA and IPC EMG were recorded during high-permeability pulmonary edema in six capsaicin-desensitized lambs. The effects of an intravenous injection of 0.05 ml/kg halothane were studied. Each halothane injection was followed by a recording period of at least 30 min. Because previous studies have shown that in one-third of the lambs expiratory upper airway closure appeared only after two (and even 4 in 1 lamb) halothane injections (10, 11), an additional injection was given if the previously described expiratory upper airway closure did not appear. Measurement of arterial blood gases (model 1306, Instrumentation Laboratory, Lexington, MA) was performed in baseline conditions and 15 and 30 min after each injection. Results were corrected for rectal temperature (model 6500, Mon-a-Therm, St. Louis, MO) (3).

Pulmonary edema was evaluated by macroscopic observation during systematic autopsy and by measurement of lung-to-body weight and wet-to-dry lung weight ratios, as described previously (11).

Data Analysis

Minute ventilation (Ve), VT, breathing frequency (f), and duty cycle (Ti/Tr) were computed for each breath in all lambs. Intravenous bolus injection of capsaicin. Averages were calculated over 15 s during baseline recordings and at preset intervals during the experimental procedure, i.e., at the 30th, 60th, and 120th s in intact lambs and at the 30th, and 60th s after the start of capsaicin injection in capsaicin-desensitized lambs.

High-permeability pulmonary edema in capsaicin-desensitized lambs. Averages were calculated over 15 s during baseline recordings and at preset intervals during the experimental procedure, i.e., 15 and 30 min after each halothane injection in capsaicin-desensitized lambs. We looked for expiratory airflow braking on the instantaneous airflow traces during the above-defined 15-s epochs in all lambs. Simultaneously, TA and IPC EMG were carefully observed throughout the experimental procedure. The percentage of breaths...
with expiratory airflow braking and the percentage of breaths with expiratory TA EMG were calculated during the above-defined 15-s epochs in each lamb. Expiratory IPC EMG was quantified by averaging the maximal voltage of the integrated signal over the same 15-s epochs and expressing the average values as a percentage of the average value during baseline room air breathing (taken as 100%). Finally, average values for all lambs as a whole were calculated for statistical analysis (19).

Statistical analysis. Above-defined averaged values were compared by analysis of variance for repeated measures over the 30-min period after the first halothane injection (further analyses were impossible because of the few remaining lambs), completed by contrast comparison when appropriate (SuperANOVA 1989, Abacus Concepts, Berkeley, CA). Lung-to-body weight and wet-to-dry lung weight ratios were compared with historical control lambs \( n = 10 \), mean age 19 ± 11 (SD) days, weight 7.4 ± 2.5 kg by a Mann-Whitney U-test.

RESULTS

Intravenous Bolus Injection of Capsaicin

Baseline room-air breathing. Respiratory parameter values of both intact and capsaicin-desensitized lambs are presented in Fig. 1. Whereas a significant increase in \( \text{Ti}/\text{TT} \) and \( \dot{V}_{\text{E}} \) was observed in capsaicin-desensitized lambs compared with intact lambs, no significant difference was observed for \( V_{r} \) and \( f \) (Mann-Whitney U-test). In both groups there was no expiratory TA EMG activity, but phasic expiratory IPC EMG activity was present in capsaicin-desensitized lambs.

Response to capsaicin bolus injection in intact lambs. The intravenous injection of 5–10 µg/kg capsaicin led to a biphasic response consisting of an apnea immediately followed by rapid shallow breathing (Fig. 2A). 

APNEA. Immediately after the injection, a central apnea occurred (7 of 7 lambs), lasting 5–8 s (7 ± 1.4 s) after injection of 5 µg/kg and 5.5–11 s (8.7 ± 2.3 s) after injection of 10 µg/kg. During this apnea, a continuous TA EMG activity was present, either during the first 3 s of apnea (3 of 7 lambs) or throughout the apnea (4 of 7 lambs). Simultaneously, a continuous IPC EMG activity was observed throughout the apnea (6 of 7 lambs) or when TA EMG decreased 3 s after apnea onset (1 of 7 lambs). At this moment, lambs appeared conscious, with open eyes, and did not look uncomfortable. All lambs exhibited a swallowing movement at the end of the apnea and often moved just before breathing resumed.

RAPID SHALLOW BREATHING. After breathing resumed all intact lambs (after either 5 or 10 µg/kg capsaicin)
exhibited a rapid shallow breathing characterized by significantly increased \( V^E \) (\( F = 5.12, P < 0.016 \)) and \( f \) (\( F = 5.09, P < 0.017 \)) and decreased \( Vr \) (\( F = 8.32, P < 0.003 \)) (Fig. 1A). All respiratory parameter values returned to near baseline conditions within 120–240 s. TA and IPC EMG were unfortunately not available during rapid shallow breathing because high \( f \) interfered with EMG recording. During rapid shallow breathing, lambs were conscious, we did not observe any abnormality in tonus or any sign of discomfort.

Capsaicin desensitization. Injections of 25 or 50 mg/kg capsaicin given while the lambs were under general anesthesia were well tolerated. In the days after the desensitization procedure, lambs appeared indifferent to pain and hunger; they lost appetite to the point that it was decided to bottle-feed the animals systematically. These abnormalities were much more apparent after 50 mg/kg capsaicin diluted in DMSO than after 25 mg/kg capsaicin diluted in Tween 20.

Response to capsaicin bolus injection in capsaicin-desensitized lambs. The intravenous injection of capsaicin, even at 50 µg/kg, did not lead to the previously described sustained expiratory upper airway closure after the first halothane injection; this lamb did not receive further injections. Five of the six remaining lambs received two doses of 0.05 ml/kg. Two lambs died soon after this second injection. A third injection was given to the last three lambs; only one survived this last injection and did not exhibit any sustained expiratory upper airway closure.

Baseline room-air breathing. Respiratory parameters are presented in Fig. 3. None of the lambs exhibited any expiratory airflow braking (6 of 6 lambs) or expiratory TA EMG (5 of 5 lambs; Fig. 4). Non-respiratory-related bursts of TA EMG were consistently observed with swallows (5 of 5 lambs). Expiratory IPC EMG was observed in five of five lambs (Fig 5). Arterial blood-gas values are presented in Fig. 6.

High-permeability pulmonary edema. Halothane injections induced a high-permeability pulmonary edema, as evidenced by the characteristic macroscopic findings observed at systematic postmortem examination. Increased lung water content was confirmed by significant increase in both lung wet-to-dry weight and lung-to-body weight ratios (\( n = 6 \); Fig. 7).

Early transient response. Immediately after the first halothane injection, a 10- to 20-s hypopnea (2 of 6 lambs) was observed (individual \( V^E < 50\% \) mean \( V^E \) in the minute preceding injection) with expiratory airflow braking, expiratory TA EMG, and increased IPC expiratory EMG (1 of 2 lambs). There was no early apnea except after last injection, which was often immediately followed by an apnea lasting 25–280 s (4 of 6 lambs).
lambs) and occurring ~20 min before death (3 of 4 lambs).

SUSTAINED RESPONSE. After this early transient response, there was a significant decrease in VT, whereas f tended to increase (Fig. 3). After the first halothane injection, five of six desensitized lambs did not exhibit any consistent expiratory airflow braking, expiratory TA EMG, or increased IPC EMG, such as that observed in intact lambs (8). After the second halothane injection, two of five lambs exhibited irregular breathing, often with very prolonged expiratory time, expiratory airflow braking, expiratory TA EMG, and increased IPC EMG; breathing patterns became more and more irregular and progressively led to death 8 and 12 min after the second halothane injection. To ensure that lack of expiratory airflow braking was not due to insufficient pulmonary edema in the three remaining lambs, a third injection of halothane was performed. One of these three lambs died immediately. The second lamb began to exhibit irregular breathing with prolonged expiratory airflow braking 15 min after this third injection and died 5 min later. The remaining lamb did not exhibit any expiratory airflow braking, TA EMG, or increased IPC EMG over the 30 min after the third injection and was killed thereafter. Blood-gas analyses revealed progressive acidosis and hypoxia.

DISCUSSION

The results of the present study provide unique insight on pulmonary C-fiber function in nonsedated, conscious lambs, especially with regard to the mechanisms linking pulmonary edema, pulmonary C fibers, and upper airway dynamics in lambs. Our results are the first 1) to describe pulmonary chemoreflex triggered by intravenous bolus injection of capsaicin in nonsedated lambs, 2) to demonstrate that capsaicin desensitization abolishes the ventilatory response to capsaicin in the newborn lamb, presumably due to loss
to show that capsaicin desensitization inhibits the active expiratory upper airway closure normally observed during halothane-induced pulmonary edema in lambs. These results suggest that C fibers play a major role in the mechanisms implicated in the upper airway response to pulmonary edema in newborn mammals.

**Pulmonary Chemoreflex in Intact Newborn Lambs**

Capsaicin, 8-methyl-N-vanillyl-6-norenamide, is a well-known stimulant of unmyelinated C fibers (5). Pulmonary C-fiber stimulation by intravenous capsaicin leads to a biphasic response consisting of an apnea followed by rapid shallow breathing similar to the classic pulmonary chemoreflex. This reflex has already been studied in anesthetized adult rabbits, cats (26), dogs (7, 9), and sheep (8) and in nonsedated adult dogs (6), sheep (9), and humans (27). Furthermore, alterations in breathing pattern or a bronchoconstriction reflex due to pulmonary C-fiber stimulation have been studied in anesthetized newborn mammals. Results from those previous studies did not yield a clear message for the neonatal period. Although alterations in breathing pattern were present in piglets (14), they were reported to be weak or absent in kittens (17) and newborn rabbits (12). Furthermore, whereas a first study was unable to show a bronchoconstriction reflex in piglets (14), it was more recently shown to be present in pups and piglets (2). The present study is the first to describe a pulmonary chemoreflex in nonsedated newborn mammals. Our results establish that a pulmonary chemoreflex can be consistently triggered by intravenous bolus injection of 5–10 µg/kg capsaicin in newborn lambs, suggesting that pulmonary C fibers are functionally mature at birth and can be involved in neonatal respiration.

**Capsaicin Desensitization**

Neonatal injection of very high doses of capsaicin is well known to functionally ablate C fibers in small rodents (16). It has become a useful tool to abolish C fibers and study their role in many organs and under different physiological conditions in rats, rabbits and guinea pigs (5). In larger mammals such as cats and dogs, a local application of capsaicin on the vagus nerve is used to silence bronchopulmonary C fibers (13, 25). Although this elegant method has the advantage to allow studies in animals at any age, its application remains difficult in nonsedated conscious animals and has been associated with a high mortality rate in some studies (13). The present results suggest that one subcutaneous injection of 25 mg/kg capsaicin under general anesthesia and maximal bronchodilation is a well-tolerated and efficient method to desensitize pulmonary C fibers “permanently,” i.e., at least for several days, in newborn lambs.

**High-Permeability Pulmonary Edema in Capsaicin-Desensitized Lambs**

In previous studies, we had shown that both high-permeability and hemodynamic pulmonary edema led
upper airway closure during pulmonary edema, we showed that decreasing lung volume did not trigger any expiratory upper airway closure (10). This result led us to examine whether C-fiber-ending activation by an excess of lung water could be responsible for expiratory upper airway closure during pulmonary edema in lambs.

Excess lung water and C-fiber endings. It is widely accepted that pulmonary C-fiber endings are stimulated by excess lung water (1, 21, 23). In decerebrate adult cats, either pulmonary artery capsaicin injection or pulmonary vascular congestion through blood infusion led to a pulmonary chemoreflex. This response was abolished in either condition once cats had undergone C-fiber ablation by application of capsaicin on both vagus nerves, which strongly suggests that pulmonary C fibers are stimulated by pulmonary congestion in adult cats (13).

C-fiber endings and upper airway dynamics. It has long been demonstrated that pulmonary C fibers interact with upper airway dynamics. An early study demonstrated that pulmonary C-fiber stimulation by intravenous phenylidiguanide injection led to an increase in expiratory laryngeal resistance in anesthetized adult cats and rabbits (26). More recently, it has been shown that activation of pulmonary C fibers through pulmonary vascular congestion induced phasic expiratory TA EMG in decerebrate cats (15). Our results in nonsedated lambs, demonstrating that intravenous bolus injection of capsaicin leads to continuous TA EMG during part of or throughout consequent central apnea, are in agreement with those results. Furthermore, as demonstrated by previous observations of increased nare dilator muscle activity in dogs (4), the effect of activated pulmonary C fibers on upper airway dynamics is probably not limited to laryngeal muscles. In agreement with this hypothesis, the present results show for the first time that IPC EMG is increased after pulmonary C-fiber activation. Hence, it appears that upper airway dynamics can be modulated by pulmonary C-fiber activation through their effects on several upper airway muscles.

Excess lung water, C-fiber endings, and upper airway dynamics in the neonatal period. Despite the importance of upper airways, especially the larynx, in neonatal respiration, the link between pulmonary edema, upper airway dynamics and pulmonary C fibers has not yet been demonstrated in newborn mammals. Results of the present study clearly demonstrate in newborn lambs that triggering of an expiratory upper airway closure during pulmonary edema can be prevented by C-fiber silencing through systemic neonatal capsaicin pretreatment. In agreement with previous data on pulmonary C-fiber function in adult mammals, the present results strongly suggest that excess lung water in newborn lambs activates C-fiber endings, which in turn reflexly leads to expiratory upper airway closure.

As for vagotomized lambs (10), a striking difference was observed between intact lambs that consistently exhibited expiratory upper airway closure after 0.05–0.075 ml/kg halothane injection (11, 19) and capsaicin-
desensitized lambs, in which none, except for one, exhibited any sustained expiratory upper airway closure. This strongly suggests that vagal C fibers are involved in the expiratory upper airway closure response to halothane-induced pulmonary edema. However, in the present study, the highest doses of halothane led to death preceded by a short period of dramatic expiratory upper airway closure with progressively developing fatal apneas in four of five lambs. As discussed in our companion paper for vagotomized lambs, the reason for this dramatic observation is not obvious. Mechanism(s) responsible might result from 1) central origin without implication of pulmonary receptors, 2) stimulation of rapidly adapting receptors (20–22), sympathetic receptors or a few C fibers surviving desensitization, and 3) untoward consequences of repeated intravenous injections of halothane.

Conclusion

The present series of experiments confirm that C-fiber endings are functionally mature in newborn lambs. Furthermore, the active expiratory upper airway closure consistently observed during pulmonary edema in nonsedated lambs appears to be mainly associated to C-fiber-ending stimulation. This does not, however, rule out the potential additional contribution of other pulmonary receptors such as rapidly adapting receptors, which have been previously shown to be activated during pulmonary edema.

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