Consequences of capsaicin treatment on pulmonary vagal reflexes and chemoreceptor activity in lambs

VÉRONIQUE DIAZ, JULIE ARSENAULT, AND JEAN-PAUL PRAUD
(With the Technical Assistance of Bruno Gagné)

Pulmonary Research Unit, Departments of Pediatrics and Physiology,
Université de Sherbrooke, Québec, Canada J1H 5N4

Received 3 June 1999; accepted in final form 12 May 2000

Diaz, Véronique, Julie Arsenault, and Jean-Paul Praud. Consequences of capsaicin treatment on pulmonary vagal reflexes and chemoreceptor activity in lambs. J Appl Physiol 89: 1709–1718, 2000.—The aim of this study was to test the hypothesis that capsaicin treatment in lambs selectively inhibits bronchopulmonary C-fiber function but does not alter other vagal pulmonary receptor functions or peripheral and central chemoreceptor functions. Eleven lambs were randomized to receive a subcutaneous injection of either 25 mg/kg capsaicin (6 lambs) or solvent (5 lambs) under general anesthesia. Capsaicin-treated lambs did not demonstrate the classical ventilatory response consistently observed in response to capsaicin bolus intravenous injection in control lambs. Moreover, the ventilatory responses to stimulation of the rapidly adapting pulmonary stretch receptors (intrathoracic water instillation) and slowly adapting pulmonary stretch receptors (Hering-Breuer inflation reflex) were similar in both groups of lambs. Finally, the ventilatory responses to various stimuli and depressants of carotid body activity and to central chemoreceptor stimulation (CO₂ rebreathing) were identical in control and capsaicin-treated lambs. We conclude that 25 mg/kg capsaicin treatment in lambs selectively inhibits bronchopulmonary C-fiber function without significantly affecting the other vagal pulmonary receptor functions or that of peripheral and central chemoreceptors.

Contrary to previous beliefs, it is now apparent that bronchopulmonary vagal C fibers are capable of functioning in newborn mammals (1, 10, 14). However, their exact role in the neonatal control of ventilation has yet to be described. Neonatal systemic injection of high doses of capsaicin has been used in small rodents, such as rats, rabbits, and guinea pigs, to block C fibers in various organs and to study their role in different experimental conditions (4). Using a similar procedure in lambs, our laboratory has recently shown that capsaicin treatment inhibits expiratory laryngeal airflow braking normally observed during pulmonary edema, suggesting a role for C-fiber endings in triggering the active expiratory laryngeal closure in neonates (10).

Potential importance of this result stems from the crucial role of this mechanism (clinically manifested by an expiratory grunting) for preventing hypoxia and acidosis in the preterm newborn with respiratory distress syndrome (13). However, there is yet no evidence that capsaicin treatment does not affect the function of other vagal afferent fibers originating from the lung, i.e., from the rapidly and slowly adapting pulmonary stretch receptors.

Previous results from a few studies conducted in adult rats treated by capsaicin neonatally have implicated C fibers in the ventilatory responses to peripheral (2, 9, 17) and central (26) chemoreceptor stimulation. Neonatal capsaicin treatment has also been shown to severely decrease the population of unmyleinated fibers in the carotid sinus nerve in adult rats (2). However, the effects of capsaicin treatment on chemoreceptor function in the neonatal period, in which postnatal resetting of peripheral chemoreceptors takes place, are totally unknown.

The aim of this study conducted in conscious lambs was twofold: 1) to test the hypothesis that the inhibition of respiratory reflexes after neonatal capsaicin treatment is restricted to those originating from bronchopulmonary C-fiber endings and does not alter the respiratory reflexes originating from the other vagal pulmonary receptors; and 2) to elucidate the contribution of C fibers within the peripheral and central chemical control of ventilation in the neonatal period in lambs.

MATERIALS AND METHODS

Animals

Eleven lambs were involved in the study. All lambs were born at term by spontaneous vaginal delivery and housed with their mother in our animal quarters for a few days before experiments were initiated. Lambs were randomly divided into two groups. The first group of six lambs, aged 2.8 ± 0.6 (range 2–4) days and weighing 4.9 ± 1.1 (range 3.1–5.8) kg, underwent capsaicin injection under general anesthesia. A control group of five lambs, aged 4.6 ± 0.9 (range 4–6) days and weighing 4.9 ± 0.95 (range 3.9–6.4) kg, were identical in control and capsaicin-treated lambs. We conclude that 25 mg/kg capsaicin treatment in lambs selectively inhibits bronchopulmonary C-fiber function without significantly affecting the other vagal pulmonary receptor functions or that of peripheral and central chemoreceptors.

Address for reprint requests and other correspondence: J.-P. Praud, Pulmonary Research Unit, Depts. of Pediatrics and Physiology, Université de Sherbrooke, Québec, Canada J1H 5N4 (E-mail: jpraud01@courrier.usherbro.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
received the same volume of solvent under general anesthesia. The protocol of the study was approved by our institution’s ethics committee for animal research.

Capsaicin Treatment

Aseptic surgery and capsaicin or vehicle injections were performed under general anesthesia (2% ethrane-40% N2O-58% O2) after premedication by an intramuscular injection of ketamine (10 mg/kg).

An arterial catheter was first inserted into the brachial artery, and all lambs were given atropine (0.2 mg/kg), buprenorphine (5 μg/kg), and ketamine (10 mg/kg) intravenously to prevent bronchospasm and pain. Capsaicin (25 mg/kg) (Sigma Chemical, St Louis, MO) was diluted in 10% Tween 80 and 10% ethanol and then in distilled water up to 10 ml for each injected lamb. The first six lambs were given two subcutaneous injections of 12.5 mg/kg of capsaicin at 10-min intervals. The five control lambs were given 10 ml of vehicle without capsaicin in two subcutaneous injections. Heart rate, arterial blood pressure, transcutaneous oxygen saturation (SaO2), and expired CO2 were continuously monitored throughout the procedure. Anesthesia was continued as long as heart rate and/or mean arterial blood pressure was 1.5-fold higher than baseline values and at least 30 min after the second capsaicin injection.

An intramuscular injection of long-acting penicillin and gentamicin was systematically given at the end of surgery. Each lamb was then allowed 2–4 days with its ewe to recover from surgery before the first examination of pulmonary reflexes.

Experimental Preparation

Just before the experiment, each lamb was intubated under local anesthesia (2% lidocaine) with a size 5 cuff endotracheal tube. A polyvinyl 5-Fr catheter was introduced in the endotracheal tube beyond 1 cm of its tip for tracheal pressure measurement, gas sampling, or water injection (see Ventilatory responses to stimulation of pulmonary vagal receptors). Furthermore, a catheter was introduced into the superior vena cava through the superficial jugular vein under local anesthesia (2% lidocaine). The lamb was comfortably positioned in a sling with loose restraints. The end of the tracheal tube was attached to a heated size 0 pneumotachograph (model 21070B plus model 8815A respiratory integrator, Hewlett-Packard, Waltham, MA). The inspiratory gas could be switched from air to various gas mixtures within <1 s (model 21049 and P314 Collins valves). A mass spectrometer (MGA-1100, Perkin-Elmer, Pomona, CA) was used to measure the inspired fraction of O2 (FIo2) and to monitor end-tidal CO2 at the distal tip of the endotracheal tube. Airflow, tracheal pressure, and tracheal O2 and CO2 fractions were recorded on an Apple microcomputer (Power Macintosh 7200/120) by using data-acquisition software (AcqKnowledge 3.2, Biopac Systems, Santa Barbara, CA) and were stored on CD for further analysis. In addition, the airflow was fed into an IBM-compatible microcomputer at a 40-Hz sampling rate and analyzed by using a custom-designed software.

Design of the Study

The experiments were divided into two series. The first series of experiments was performed 2–4 days after surgery to study the ventilatory responses to stimulation of the pulmonary vagal receptors. The second series of experiments was performed at 12–13 days of age to study central and peripheral chemoreceptor function. All experiments (except for measurement of Hering-Breuer reflex, see SLOWLY ADAPTING PULMONARY STRETCH RECEPTORS (HERING-BREUER REFLEX) below) were performed in nonsedated conscious lambs and began with a 3-min baseline recording. Ambient temperature was kept between 20 and 22°C and humidity between 50 and 70% throughout the experiments.

Ventilatory responses to stimulation of pulmonary vagal receptors. C fibers (intravenous capsaicin injection). After baseline recording, lambs were first given an intravenous (iv) bolus injection of vehicle, i.e., Tween 80 + 10% ethanol, which is the equivalent dose for 50 μg/kg capsaicin. This was followed by capsaicin injections at increasing doses (5, 10, 25, and 50 μg/kg), beginning with two injections of 5 μg/kg for control lambs and two injections of 10 μg/kg for capsaicin-desensitized lambs. Injections (1-ml volume) were given at 5-min intervals, and each dose was repeated once. The ventilatory response was judged significant when a 10-s apnea or a threefold increase in breathing frequency (f) was observed for at least 2 min. The f, tidal volume (VT), and minute ventilation (Ve) were averaged over three respiratory cycles that were the end of the baseline recording, and at the 30th, 60th, and 120th s after each injection.

RAPIDLY ADAPTING PULMONARY STRETCH RECEPTORS (COUGH REFLEX). After baseline recording, airflow was recorded during tracheal injection of water through the catheter placed in the endotracheal tube. One milliliter of water was injected over 2 s, immediately followed by a slow flush of 1 ml of air over 2 s. The number of cough movements and apnea duration after water injection was calculated. Two water injections were performed at 5-min intervals in all lambs.

SLOWLY ADAPTING PULMONARY STRETCH RECEPTORS (HERING-BREUER REFLEX). All lambs received a sedation by 100 mg/kg chloral hydrate per os before this last experiment of the series. The Hering-Breuer reflex was induced by occluding the endotracheal tube at the end of a tidal inspiration, as previously reported (3). The strength of the Hering-Breuer reflex was quantified by calculating the inhibitory ratio (IR) as follows. Control expiratory time was calculated from the airflow recording and averaged for the three breaths preceding the end-inspiratory occlusion. The expiratory time of the occluded breath was measured from the expiratory zero-flow crossing and rise in airway opening pressure to the onset of negative deflection on the airway opening pressure trace. Hence, IR corresponded to expiratory time of the occluded breath divided by control expiratory time.

Ventilatory responses to modifications of afferent activity from peripheral chemoreceptors. These experiments were performed at 12–13 days of life in all lambs, i.e., at a time when postnatal resetting of peripheral chemoreceptor function is largely completed (5). Absence of ventilatory response to capsaicin iv bolus injection (compared with 6 control lambs aged 12 days) was confirmed in capsaicin-treated lambs just before these experiments.

TRANIENT VENTILATORY TESTS. As previously reported (6), peripheral chemoreceptor sensitivities to O2 and CO2 were respectively assessed by using transient N2 and CO2 ventilatory tests adapted from the experiment originally described by Dejours (7, 8). The transient tests are based on the concept that the immediate ventilatory response, within 10 s after a step change in arterial PO2 and arterial PCO2, reflects the activity of peripheral chemoreceptors. After 3 min of baseline room-air breathing, the inhaled gas was randomly switched to room air (air test), pure N2 (N2 test), or 0.13 fractional inspired CO2/0.21 Fio2 (CO2 test) for three tidal breaths. Breath-by-breath changes in Ve, end-tidal PO2 (PETO2), and end-tidal PCO2 (PETCO2) measurements were obtained within 15 s before the transient ventilatory stimulus and within 15 s
after the stimulus. The procedure was repeated randomly until each test had been performed twice in each animal with a 2-min rest period between consecutive stimuli. Transient ventilatory tests were accepted as valid if the pretest baseline was stable and free of visible oscillations and if breathing was not interrupted by disturbances such as sighs, agitation, or apneas.

The ventilatory response to each stimulus was expressed as $$\Delta V_e$$ (peak $$V_e$$ – pretest $$V_e$$), the magnitude of the hypercapnic stimulus as $$\Delta PET_{CO_2}$$ (peak $$PET_{CO_2}$$ – pretest $$PET_{CO_2}$$), and the magnitude of the hypoxic stimulus as $$\Delta PET_{O_2}$$ (pretest $$PET_{O_2}$$ – nadir $$PET_{O_2}$$). Peripheral chemoreceptor sensitivity to hypercapnia was determined by the ratio $$\Delta V_e/\Delta PET_{CO_2}$$ and peripheral chemoreceptor sensitivity to hypoxia by the ratio $$\Delta V_e/\Delta PET_{O_2}$$ (in ml·min⁻¹·kg⁻¹·Torr⁻¹) (6).

**KCN and Dopamine Injections.** Ventilatory response to carotid body stimulation by KCN iv injection (5) and to carotid body inhibition by dopamine iv injection (16) was assessed as follows. After 3 min of baseline room-air breathing, a bolus of 0.1 mg/kg KCN, 5 µg/kg dopamine, 10 µg/kg dopamine, or saline through a catheter positioned in the superior vena cava. Saline was added as needed to keep the volume of each bolus to 1 ml, and 1 ml of saline was systematically used as a flush. Each injection was performed twice in a random order in each lamb and at 2-min intervals.

**Steady-State Hypoxia and Dejours Tests.** The ventilatory response to a 15-min hypoxic hypercapnic period was studied in all lambs. Furthermore, the effect of peripheral chemoreceptors during hypoxic breathing was studied by performing transient pure $$O_2$$ inhalations, thereby silencing peripheral chemoreceptor activity. As previously described (20), we recorded baseline ventilation for 3 min, during which time the Dejours test was performed at the 1- and 2-min marks. Each Dejours test consisted of inhalation of pure $$O_2$$ during five breaths (7). After the initial 3 min of room-air baseline recording, we abruptly switched the animal to 0.08 FIO2 for a breath (7). After the initial 3 min of room-air baseline breathing, when $$FETCO_2$$ reached 6, 8, and 10%, and 1 and 2 min after the return to room-air breathing. Furthermore, apnea duration after each Dejours test was measured.

Ventilatory responses to stimulation of central chemoreceptors. After 3 min of baseline room-air breathing, lambs were abruptly switched to a rebreathing bag filled with 50 ml/kg of 0.05 CO2-0.95 O2 gas mixture. Respiratory parameters were recorded until end-tidal fractional CO2 ($$FEtCO_2$$) reached 10%. The lamb was then switched back to air for a 2-min recording (21). Breathing parameters were averaged over 15 s and were analyzed 1 min before the test during baseline room-air breathing, when $$FEtCO_2$$ reached 6, 8, and 10%, and 1 and 2 min after return to room air.

**Statistical Data Analysis**

Group means are reported as means ± SD. When the same test was repeated in one lamb, results were first averaged in each lamb. Group mean values were analyzed by a Mann-Whitney U-test for unpaired comparisons (Statview 4.01, Abacus Concepts, Berkeley, CA) or by analysis of variance for repeated measures completed by contrast comparison when repeated measures were obtained in the same lamb (SuperANOVA 1989, Abacus Concepts). Regression analysis was used for the CO2 rebreathing test. A value of $$P < 0.05$$ was considered significant.

**RESULTS**

**Capsaicin Treatment**

After the animals were injected with 25 mg/kg capsaicin under general anesthesia, we observed a rise in heart rate and arterial blood pressure without any decrease in transcutaneous $$SAO_2$$. Clonic movements were observed in two of six lambs 25–30 min after capsaicin injection. These lambs were still under general anesthesia, with normal oxygenation ($$SAO_2 > 96$$%) and mean arterial blood pressure between 47 and 66 mmHg. Seizures were not electrically proven (no electroencephalogram recording), and clonic movements ceased spontaneously within 2 min. All lambs awoke without any obvious abnormality after anesthesia discontinuation. Lambs seemed, however, somewhat indifferent to pain in the days after the desensitization procedure. Subcutaneous injection of solvent in control lambs did not lead to any immediate change in heart rate, arterial pressure, or transcutaneous $$SAO_2$$. 

**Ventilatory Responses to Stimulation of Pulmonary Vagal Receptors**

Ventilatory response to iv capsaicin at 4–8 days of age. Baseline Room-Air Breathing. Respiratory parameter values in baseline conditions at 6 and 12 days of life were not significantly different in capsaicin-treated vs. control lambs (Table 1).

**Control Lambs.** The iv injection of 5–10 µg/kg capsaicin led to a biphasic response consisting of an apnea immediately followed by tachypnea (Fig. 1A). One lamb exhibited a threefold increase in f in response to 5 µg/kg and hence did not receive higher doses. A central apnea lasting 2–4.5 s was observed in four of the five lambs (7 of 10 injections) immediately after 5 µg/kg iv capsaicin injection; 10 µg/kg iv capsaicin injection was followed by a central apnea lasting 2.4 to 8.1 s in three of the four remaining lambs (5 of 8 injections) (Table 2). During the apnea, lambs were
conscious and calm, with eyes opened. At the end of the apnea, most lambs exhibited swallowing movements and a bout of agitation during a few seconds (<5 s). After breathing resumption, all control lambs (after either 5 or 10 μg/kg capsaicin) exhibited a period of tachypnea lasting at least 60 s after 5 μg/kg and more than 120 s after 10 μg/kg that was characterized by a significant increase in Ve and f but a decreasing tendency in Vt (Fig. 2A). During tachypnea, lambs were conscious and often exhibited neck hyperextension with their mouth open. All respiratory parameters returned to baseline values within 120–240 s. Vehicle iv injection did not lead to apnea or variation in breathing parameters in any of the control lambs.

CAPSAICIN-TREATED LAMBS. One of the six lambs exhibited a threefold increase in f for >1 min after 25 μg/kg capsaicin and for >2 min after 50 μg/kg capsaicin. Although its response to 10 μg/kg was much weaker than that in control lambs, this lamb was considered not to be fully C-fiber desensitized and was hence excluded from further analyses.

Injections of 10 and 25 μg/kg iv capsaicin did not lead to any apnea in the five remaining lambs, except in one lamb that exhibited a 7-s apnea after only one injection of 25 μg/kg capsaicin (Table 2). No tachypnea was observed after 10 or 25 μg/kg iv capsaicin injection (Fig. 1B). Injection of 50 μg/kg iv capsaicin led to an apnea lasting 2–6 s in five lambs (6 of 10 injections), without any other significant change in respiratory parameters thereafter (Fig. 2B). Vehicle iv injection did not lead to apnea or variation in breathing parameters in any of the treated lambs.

Ventilatory response to iv capsaicin at 12–13 days of age. The ventilatory response to capsaicin injection observed in the six control lambs, aged 12 days, was again severely blunted in capsaicin-treated lambs (Figs. 1C and 3), with virtually no increase in f and no decrease in Vt. On the contrary, a slight increase in Vt was observed. Characteristics of apneas after capsaicin bolus iv injection are reported in Table 2 for control and capsaicin-treated lambs.

Stimulation of rapidly adapting pulmonary stretch receptors. Intratracheal injection of 1 ml water consistently led to 6 ± 2.1 (range 2–19) cough movements in the five capsaicin-pretreated lambs, followed, in all lambs (5 of 9 injections), by an apnea lasting 4.9 ± 2.1 (range 2.5–8) s. The five control lambs consistently exhibited 2.7 ± 1.2 (range 1–4) cough movements, followed by an apnea lasting 5.7 ± 4.3 (range 2–12) s in four lambs (7 of 10 injections). The duration of apnea was not significantly different in capsaicin-treated lambs compared with control lambs. However, because of a high number of cough efforts in one lamb, the number of cough efforts in the five capsaicin-treated lambs as a whole was significantly increased compared with that in control lambs (P < 0.05).

### Table 2. Characteristics of apneas following capsaicin bolus intravenous injection in control and capsaicin-treated lambs at 6 and 12 days of age

<table>
<thead>
<tr>
<th>Capsaicin, μg/kg</th>
<th>Age 6 Days</th>
<th>Age 12 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Apneas/injections</td>
<td>7/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Lambs with apneas, no.</td>
<td>4/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>2–6</td>
<td>6.5</td>
</tr>
<tr>
<td>10</td>
<td>Apneas/injections</td>
<td>5/8</td>
</tr>
<tr>
<td>Lambs with apneas, no.</td>
<td>3/4</td>
<td>0/5</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>2.5–8</td>
<td>3.5–8.5</td>
</tr>
<tr>
<td>25</td>
<td>Apneas/injections</td>
<td>1/2</td>
</tr>
<tr>
<td>Lambs with apneas, no.</td>
<td>1/1</td>
<td>1/5</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>4.5</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
<td>Apneas/injections</td>
<td>6/10</td>
</tr>
<tr>
<td>Lambs with apneas, no.</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Apnea duration, s</td>
<td>2–6</td>
<td>3.5–9</td>
</tr>
</tbody>
</table>

*Apneas/injections, total no. of apneas over total no. of capsaicin intravenous injections performed in the group under study; lambs with apneas, no. of lambs rendered apneic by capsaicin intravenous injection over total no. of lambs tested in the group under study.*
Stimulation of slowly adapting pulmonary stretch receptors. Occlusion of the endotracheal tube at the end of inspiration led to a prolongation of expiratory time in both groups of lambs (Fig. 4). The Hering-Breuer reflex IR was not significantly different in the five capsaicin-treated lambs (3.7 ± 0.2, range 3.4–4) compared with control lambs (4.1 ± 1.2, range 2.5–5.3).

Ventilatory Response to Modifications of Input From Peripheral Chemoreceptors

Transient ventilatory tests. The N₂ tests led to a decrease in PETO₂ (ΔPETO₂) in both intact [63 ± 8.7 (range 52–71) Torr] and capsaicin-treated lambs [69 ± 15 (range 44–83) Torr] and an increase in V̇E (ΔV̇E) in both intact [268 ± 67 (range 159–343) ml·kg⁻¹·min⁻¹] and capsaicin-treated lambs [258 ± 52 (range 177–319) ml·kg⁻¹·min⁻¹]. The ratio ΔV̇E/ΔPETO₂ was not significantly different in control lambs [4.2 ± 0.76 (range 3.2–5.2) ml·min⁻¹·kg⁻¹·Torr⁻¹] and in capsaicin-treated lambs [3.8 ± 0.4 (range 3.3–4.2) ml·min⁻¹·kg⁻¹·Torr⁻¹].

The CO₂ tests led to an increase in PETCO₂ (ΔPETCO₂) in both control [30 ± 4.9 (range 24–36) Torr] and capsaicin-treated lambs [29 ± 4.7 (range 22–35) Torr].
and an increase in $\dot{V}_E$ ($\Delta\dot{V}_E$) in both intact [122 ± 17 (range 109–149) ml·kg$^{-1}$·min$^{-1}$] and capsaicin-treated lambs [112 ± 85 (range 19–210) ml·kg$^{-1}$·min$^{-1}$]. The ratio $\Delta\dot{V}_E/\Delta P_{ETCO2}$ was not significantly different in control lambs [4.2 ± 0.6 (range 3.7–5.2) ml·min$^{-1}$·kg$^{-1}$·Torr$^{-1}$] and in capsaicin-treated lambs [3.8 ± 2.6 (range 0.7–7.2) ml·min$^{-1}$·kg$^{-1}$·Torr$^{-1}$].

In both groups, air tests did not lead to any change in $P_{ETO2}$, $P_{ETCO2}$, or $\dot{V}_E$.

**KCN injection.** In both groups, ventilation peaked within 10 s after the iv KCN bolus injection. The $f$, $V_T$, and $\dot{V}_E$ significantly increased at the peak ventilation point but returned to baseline values 30 s after KCN injection. The increase in $\dot{V}_E$ from baseline to peak ventilation, when expressed as a function of baseline $\dot{V}_E$ [(peak $\dot{V}_E$ − baseline $\dot{V}_E$)/baseline $\dot{V}_E$], was not significantly different between control (2.7 ± 1, range 1.3–3.8) and capsaicin-treated lambs (2.8 ± 0.9, range 1.4–4) (Fig. 5).

**Dopamine injection.** Intravenous bolus injection of dopamine led to an apnea (≥3 s) in four of five capsaicin-treated lambs and four of five control lambs (Fig. 6). One intact lamb required 10 µg/kg dopamine, and one capsaicin-treated lamb required 5 µg/kg dopamine before exhibiting an apnea; 2 µg/kg was enough to trigger an apnea in all other lambs. After 10 µg/kg dopamine iv bolus injection, apnea duration was not significantly different between capsaicin-treated lambs, which exhibited apneas lasting 3, 4.5, 11, and 12 s, and control lambs, which exhibited apneas lasting 7, 10, 10, and 12 s.

**Steady-State Hypoxia and Dejours’ Test**

The ventilatory response to 0.08 $F_{I2O}$ breathing during 15 min is illustrated for both groups in Fig. 7. As previously reported (20), $\dot{V}_E$ significantly peaked during the first 3 min of hypoxia and then progressively...
decreased in both control and capsaicin-treated lambs. However, $f$, $V_T$, and $V_E$ were still above prehypoxia values at the 15-min mark of hypoxia in both groups of lambs. Moreover, both $V_T$ and $f$ clearly had an identical time course in both groups of lambs. Dejours test during hypoxia consistently led to an apnea lasting $10.1 \pm 4.5$ (range 9.2–30.8) s in control lambs (Fig. 8A) and 11.5 $\pm$ 10.2 (range 3.6–28.5) s in capsaicin-treated lambs (Fig. 8B). Apnea duration was not different between the two groups.

**Ventilatory Response to Stimulation of Central Chemoreceptors**

CO$_2$ rebreathing led to a significant increase in $f$, $V_T$, and $V_E$ from baseline room air to 8 and 10% $F_{ETCO_2}$ in both control and capsaicin-treated lambs (Fig. 9). The increase in $V_E$ from baseline to 10% $F_{ETCO_2}$, when expressed as a function of baseline $V_E$ \([10\% \text{~CO}_2 \text{~} V_E - \text{~baseline } V_E]/\text{baseline } V_E\] was not significantly different between controls ($1.4 \pm 0.6$) and capsaicin-treated lambs ($2.5 \pm 1$) (Mann-Whitney U-test). Furthermore, the slope of the regression line between $F_{ETCO_2}$ and $V_E$ was not significantly different in capsaicin-treated compared with control lambs (Fig. 9).

**DISCUSSION**

This study is the first to describe the consequences of neonatal capsaicin treatment on the ventilatory responses to stimulation of pulmonary vagal receptors and of peripheral and central chemoreceptors in conscious newborn mammals. In contrast to an abolishment of the ventilatory response to C-fiber stimulation by iv capsaicin, the striking point in this study is that the ventilatory response to stimulation of rapidly and slowly adapting pulmonary stretch receptors and of central and peripheral chemoreceptors was not decreased by neonatal capsaicin treatment. These results suggest that neonatal capsaicin treatment selectively blocks bronchopulmonary C fibers in lambs and thus appears to be a useful tool to study the involvement of these C fibers within the context of neonatal respiratory control.
Effectiveness and Duration of C-Fiber Blockade by Neonatal Capsaicin Treatment in Lambs

Neonatal systemic injections of very high doses of capsaicin have been shown to abolish C-fiber function in rodents (15). Our results, obtained from a total of nine lambs (Ref. 10 and present study), establish that a dose of 25 mg/kg capsaicin injected subcutaneously is most often sufficient to abolish C-fiber function in newborn lambs. However, the observation in one of the six treated lambs that iv capsaicin was still able to induce a significant (although decreased) ventilatory response shows that it is mandatory to verify the effectiveness of C-fiber blockade after capsaicin treatment in all lambs. Data from the present study show that C-fiber blockade after neonatal capsaicin treatment lasts at least until 12–13 days of age in lambs. Capsaicin treatment in rodents has been shown to induce an alteration of anterograde (12) and retrograde axoplasmic transport (18), presumably due to destruction of the microtubule system of C fibers. Such alterations would explain the inability of neuromediator storage and its consequences on nerve development through loss of growth factor transport (4). Accordingly, C-fiber degeneration and blockade induced by neonatal capsaicin treatment last through adulthood in rodents, and it seems reasonable to hypothesize that the same holds true in lambs. This, however, remains to be proven.

Vagal Input and Capsaicin Treatment

Rapidly adapting pulmonary stretch receptors and capsaicin pretreatment. Both rapidly adapting pulmonary stretch receptors and C fibers are presumably activated by similar stimuli, such as excess lung water (23). The ability to inhibit one type of receptors without altering the activity of the other would, therefore, be useful in better understanding the contribution of each receptor type under various conditions. A recent study in adult, anesthetized rats demonstrating that neonatal capsaicin treatment decreases responsiveness to methacholine (24) led us to suspect that rapidly adapting pulmonary stretch receptor function could be altered by neonatal capsaicin treatment in lambs. Instillation of 1 ml of water in the trachea is a rough test that can stimulate C fibers as well as rapidly adapting pulmonary stretch receptors (19). However, the persistence of cough efforts and apnea in response to water instillation into the trachea of C-fiber-silenced lambs suggests that rapidly adapting pulmonary stretch receptors are still functional after neonatal capsaicin treatment. Our results are in agreement with the recent demonstration that perineural capsaicin application leads to C-fiber silencing without affecting rapidly adapting pulmonary stretch receptors in anesthetized lambs that iv capsaicin was still able to induce a significant (although decreased) ventilatory response shows that it is mandatory to verify the effectiveness of C-fiber blockade after capsaicin treatment in all lambs. Data from the present study show that C-fiber blockade after neonatal capsaicin treatment lasts at least until 12–13 days of age in lambs. Capsaicin treatment in rodents has been shown to induce an alteration of anterograde (12) and retrograde axoplasmic transport (18), presumably due to destruction of the microtubule system of C fibers. Such alterations would explain the inability of neuromediator storage and its consequences on nerve development through loss of growth factor transport (4). Accordingly, C-fiber degeneration and blockade induced by neonatal capsaicin treatment last through adulthood in rodents, and it seems reasonable to hypothesize that the same holds true in lambs. This, however, remains to be proven.

Vagal Input and Capsaicin Treatment

Rapidly adapting pulmonary stretch receptors and capsaicin pretreatment. Both rapidly adapting pulmonary stretch receptors and C fibers are presumably activated by similar stimuli, such as excess lung water (23). The ability to inhibit one type of receptors without altering the activity of the other would, therefore, be useful in better understanding the contribution of each receptor type under various conditions. A recent study in adult, anesthetized rats demonstrating that neonatal capsaicin treatment decreases responsiveness to methacholine (24) led us to suspect that rapidly adapting pulmonary stretch receptor function could be altered by neonatal capsaicin treatment in lambs. Instillation of 1 ml of water in the trachea is a rough test that can stimulate C fibers as well as rapidly adapting pulmonary stretch receptors (19). However, the persistence of cough efforts and apnea in response to water instillation into the trachea of C-fiber-silenced lambs suggests that rapidly adapting pulmonary stretch receptors are still functional after neonatal capsaicin treatment. Our results are in agreement with the recent demonstration that perineural capsaicin application leads to C-fiber silencing without affecting rapidly adapting pulmonary stretch receptors in anesthetized lambs that iv capsaicin was still able to induce a significant (although decreased) ventilatory response shows that it is mandatory to verify the effectiveness of C-fiber blockade after capsaicin treatment in all lambs. Data from the present study show that C-fiber blockade after neonatal capsaicin treatment lasts at least until 12–13 days of age in lambs. Capsaicin treatment in rodents has been shown to induce an alteration of anterograde (12) and retrograde axoplasmic transport (18), presumably due to destruction of the microtubule system of C fibers. Such alterations would explain the inability of neuromediator storage and its consequences on nerve development through loss of growth factor transport (4). Accordingly, C-fiber degeneration and blockade induced by neonatal capsaicin treatment last through adulthood in rodents, and it seems reasonable to hypothesize that the same holds true in lambs. This, however, remains to be proven.

Vagal Input and Capsaicin Treatment

Rapidly adapting pulmonary stretch receptors and capsaicin pretreatment. Both rapidly adapting pulmonary stretch receptors and C fibers are presumably activated by similar stimuli, such as excess lung water (23). The ability to inhibit one type of receptors without altering the activity of the other would, therefore, be useful in better understanding the contribution of each receptor type under various conditions. A recent study in adult, anesthetized rats demonstrating that neonatal capsaicin treatment decreases responsiveness to methacholine (24) led us to suspect that rapidly adapting pulmonary stretch receptor function could be altered by neonatal capsaicin treatment in lambs. Instillation of 1 ml of water in the trachea is a rough test that can stimulate C fibers as well as rapidly adapting pulmonary stretch receptors (19). However, the persistence of cough efforts and apnea in response to water instillation into the trachea of C-fiber-silenced lambs suggests that rapidly adapting pulmonary stretch receptors are still functional after neonatal capsaicin treatment. Our results are in agreement with the recent demonstration that perineural capsaicin application leads to C-fiber silencing without affecting rapidly adapting pulmonary stretch receptors in anesthetized lambs that iv capsaicin was still able to induce a significant (although decreased) ventilatory response shows that it is mandatory to verify the effectiveness of C-fiber blockade after capsaicin treatment in all lambs. Data from the present study show that C-fiber blockade after neonatal capsaicin treatment lasts at least until 12–13 days of age in lambs. Capsaicin treatment in rodents has been shown to induce an alteration of anterograde (12) and retrograde axoplasmic transport (18), presumably due to destruction of the microtubule system of C fibers. Such alterations would explain the inability of neuromediator storage and its consequences on nerve development through loss of growth factor transport (4). Accordingly, C-fiber degeneration and blockade induced by neonatal capsaicin treatment last through adulthood in rodents, and it seems reasonable to hypothesize that the same holds true in lambs. This, however, remains to be proven.
adult dogs (25). Again, the dose of capsaicin used for neonatal treatment and/or the technique used to assess rapidly adapting pulmonary stretch receptors function and/or species difference may account for the differences observed between this study and certain other previous studies.

**Slowly adapting pulmonary stretch receptors and capsaicin treatment.** The Hering-Breuer inflation reflex assesses the influence of vagal input from the slowly adapting pulmonary stretch receptors on both ventilatory timing and drive. Contrary to adults, newborns and infants have an active Hering-Breuer inflation reflex that may facilitate the rapid respiratory rate and favor maintenance of an adequate resting lung volume (3). Vagal inputs from slowly adapting pulmonary stretch receptors are likely to play a significant role in eupneic breathing during the first year of life (22). The present data show that neonatal capsaicin treatment does not significantly affect the Hering-Breuer inhibitory reflex in newborn lambs and suggest that slowly adapting pulmonary stretch receptors are preserved. Our results are in agreement with previous observations that vagal perineural capsaicin does not modify the Hering-Breuer reflex in anesthetized adult dogs (25).

**Carotid Body Function and Capsaicin Treatment**

Substance P (SP)-like material has been repeatedly identified in the afferent pathway from peripheral chemoreceptors, including the carotid body, sinus nerve, petrosal ganglion, and nucleus tractus solitarius. A variety of results suggests that SP plays a role in the ventilatory response to hypoxia (see Ref. 9 for review). Accordingly, neonatal capsaicin treatment, known to deplete C-fiber stores of SP, has been shown to significantly decrease the ventilatory response to the usual stimuli of peripheral chemoreceptors such as hypoxia or NaCN in anesthetized adult rats (2, 17) and in unanesthetized adult rabbits (11) and rats (9). Furthermore, the population of unmyelinated fibers in the carotid sinus nerves of capsaicin-treated rats has been reported to be severely reduced (2).

Reconciliation of the above results with observations made in the present study is not straightforward. Indeed, in our hands, peripheral chemoreceptor stimulation (or inhibition) using various means led to what appears to be a similar increase (or depression) in ventilation in control and capsaicin-treated lambs. It might be that unmyelinated fibers and SP are less important within the afferent pathway leading from peripheral chemoreceptor to respiratory centers in sheep than in rodents. However, we are not aware of any data to support or refute that hypothesis. In fact, some data in rodents also seem to give support to our findings in lambs; hence, previous studies failed to find any significant alteration in the ventilatory inhibition secondary to dopamine iv injection in anesthetized, capsaicin-treated rats (17). Furthermore, in contrast to what is observed for vagal C fibers, neonatal capsaicin treatment does not induce any noticeable depletion of SP-like material content in the carotid body in adult rabbits (11). Clearly, more work is needed to clarify the role of C fiber and SP in peripheral chemoreception. Meanwhile, our results suggest that neonatal capsaicin treatment does not significantly affect the postnatal resetting of carotid body function in lambs.

**Central Chemoreceptors and Capsaicin Treatment**

Previous studies on the effects of neonatal capsaicin treatment on central chemoreceptor function in adult rats have yielded contradictory results. From the observation of an altered ventilatory response to hypercapnia, Towle et al. (26) concluded that C fibers in the brain stem modulate the hypercapnic ventilatory response. However, alteration of the hypercapnic ventilatory response was not confirmed in a more recent study in anesthetized rats, with the difference being attributed to the use of anesthesia in the first study (9).

Results of the present study in nonsedated lambs, while limited to the neonatal period, confirm the absence of effect of neonatal capsaicin treatment on the overall ventilatory response to hypercapnia, which suggests that central chemoreceptor function is not altered.

In conclusion, in lambs, neonatal treatment by a subcutaneous injection of 25 mg/kg capsaicin appears to be a safe and efficient way to block bronchopulmonary C fibers without significantly altering the other pulmonary vagal afferent fibers, as well as peripheral and central chemoreceptors. Use of this procedure recently allowed us to demonstrate that C-fiber endings may play a central role in the respiratory adaptation of newborn infants, especially in pathological conditions. The possibility of selectively and chronically blocking bronchopulmonary C fibers in lambs will help in the further understanding of the likely important role of C-fiber input within the neonatal control of breathing.

The authors thank Dr. André Denjean for useful review of the manuscript.

Jean-Paul Praud is a scholar of the Fonds de la Recherche en Santé du Québec. This work was supported by a grant from the Centre de Recherche Clinique, Centre Universitaire de Santé de l’Estrie, Quebec, and by Medical Research Council Grant MT-15558.

Present address of V. Diaz: Service de Physiologie Respiratoire du Pr Denjean, Pavillon Beauchant, CHU de Poitiers, 350 Av. J. Coeur, 86000 Poitiers, France.

**REFERENCES**