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Two Subgroups of Lung Vagal C-Fibers with Different Vulnerabilities to Blockades by Perivagal Capsaicin and Vagal Cooling in Dogs

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Abstract

Perivagal capsaicin treatment and vagal cooling are two techniques that have been widely used to study the respiratory reflexes mediated by lung vagal C-fibers because they can block the neural conduction of unmyelinated fibers. We hypothesized that there are two subgroups of lung vagal C-fibers which have different vulnerabilities to blockades by these two techniques. To test this hypothesis, afferent activity arising from lung vagal C-fibers was recorded in 29 anesthetized, paralyzed, and artificially ventilated dogs. Afferent C-fiber activity was recorded before and after various concentrations of perivagal capsaicin treatment or before and during various temperatures of vagal cooling. Of the 89 lung vagal C-fibers studied, 73 fibers were classified as the group of "low resistance" to capsaicin, while the other 16 were classified as the group of "high resistance". The former group differed from the latter due to their afferent activity being blocked at relatively low concentrations of perivagal capsaicin and at relatively low temperatures of vagal cooling. Our results suggest that lung vagal C-fibers can be categorized into two subgroups, based upon their different blocking thresholds for perivagal capsaicin and vagal cooling. Our data may provide information for researchers to further differentiate the respiratory reflexes originating from these two subgroups of lung vagal C-fibers.

Key Words: conduction blockade, perivagal capsaicin, vagal cooling, dog

Introduction

It is well recognized that lung vagal C-fibers play an important role in evoking respiratory reflexes in response to the airway assault by inhaled irritants (5, 15, 17-20, 28) and in detecting the onset of pathophysiological conditions (2, 5, 14, 27, 29). Stimulation of these C-fiber nerve endings results in various airway reflexes including bronchoconstriction, changes in breathing pattern, and an increase in mucus secretions (5, 15, 28). These C-fiber afferents therefore are thought to be part of the airway defense mechanism (5, 29). However, the physiological properties of these pulmonary receptors are not completely understood.

Several physiological properties of lung vagal C-fibers have been documented (5, 29). In general, afferent activity arising from these sensory nerve endings is conveyed by vagal unmyelinated fibers which have a conduction velocity of less than 2 m/s. These receptors have a higher threshold for mechanical stimuli and a greater sensitivity to chemical stimuli as compared to lung myelinated afferents. Based upon these properties, previous investigators (12, 17, 19, 20, 27, 28) have employed perivagal capsaicin treatment and vagal cooling to explore the respiratory functions of lung vagal C-fibers. The former technique is based upon the fact that capsaicin, a chemical

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extract from hot pepper, appears to have selective effects on unmyelinated sensory fibers (7, 24). When injected at a relatively low dose (e.g., 1 μg/kg), capsaicin stimulates lung vagal C-fiber afferents, while having no effect on lung vagal myelinated afferents (5). Similarly, when locally or topically applied to the nerves at a high concentration (*e.g.*, 5 mg/ml), capsaicin preferentially blocks impulse propagation in unmyelinated C-fibers, but does not interfere with the neural conduction of myelinated fibers (1, 23, 24). On the other hand, the vagal cooling technique is based upon the fact that the neural conduction of myelinated fibers can be blocked by cooling the nerve trunk to 6 - 7 °C, whereas the blockade of unmyelinated fibers can only be achieved by cooling to 0 °C or below (5, 8, 13).

Since perivagal capsaicin treatment and vagal cooling are conventional techniques for the study of functions of lung vagal C-fibers, it is important to know whether their effects are universal across the whole group of C-fiber afferents. However, it is not known if these vagal afferents have subgroups that can be distinguished by their vulnerability to short term blockade of conduction by perivagal capsaicin treatment. Furthermore, it is also not clear whether these vagal afferents have different blocking thresholds in response to cold blockade. It is conceivable that the answers to these two questions can provide important information for the study of the respiratory functions of lung vagal C-fiber afferents using these two techniques. In this study, we attempted to resolve these questions.

Materials and Methods

Animal Preparations

Adult dogs (9-23 kg) were anesthetized with an intravenous (iv) injection of thiopental sodium (20 mg/kg; Abbott), followed by a combination of chloralose (50 mg/kg iv; Sigma) and urethan (500 mg/kg iv; Sigma). Supplemental doses of chloralose (15 mg/kg/hr) and urethan (150 mg/kg/hr) were administered to maintain abolition of the corneal and withdrawal reflexes during the course of the experiments. The femoral artery was cannulated for measuring arterial blood pressure. Catheters (PE-240) were inserted into the right and left atrium via the femoral vein and the left atrial appendage, respectively. During the recording of vagal action potentials, the dogs were paralyzed with pancuronium bromide (0.05 mg/kg iv; Organon Teknica). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked. Throughout the experiment, body temperature was maintained at ~36 °C by means of a servo heating blanket.

After a midline incision was made in the neck, a short tracheal cannula was inserted just below the larynx, and a midline thoracotomy was performed. The lungs were ventilated (Harvard 607) with 65% O_2 at a frequency of 16-20 cycles/min and a tidal volume (V_T) of 12-15 ml/kg; both were kept constant during each experiment. CO_2 was mixed with the inspired gas when necessary to maintain end-tidal CO_2 concentration at about 5%. The expiratory outlet of the respirator was placed under 3-4 cm of water to maintain a near normal functional residual capacity. Respiratory flow was measured with a pneumotachograph (Fleisch no. 1) connected to a pressure transducer (Gould model 17212). The flow signal was integrated to give V_T. Tracheal pressure (P_{tr}) and CO_2 concentration were measured via side taps of the tracheal cannula by a pressure transducer (Validyne MP45-28) and a capnograph (Biochem 9000), respectively. All physiological signals were recorded by a thermal array recorder (Gould TA11) and also recorded on tape (Neurocorder DR-890) for later analysis.

Recording of Afferent Activity of Lung Vagal C-fibers

Afferent activity arising from lung vagal C-fibers was recorded using techniques previously described (2, 18). Briefly, a fine afferent filament was split from the desheathed nerve trunk of the right vagus and placed on a silver recording electrode. Action potentials were amplified (Grass P511K), monitored by an audio amplifier (Grass AM8), and displayed on an oscilloscope (Gould 1425). The fine nerve filament was subdivided until the activity from a single unit was obtained. To compare the responses of C-fiber nerve endings to perivagal capsaicin treatment and to vagal cooling, the activities of two receptors from the same vagal fibers were recorded simultaneously with two electrodes and under the same experimental condition. We used lung inflations ($4 \times V_T$) as the first step to search for lung vagal C-fibers which usually have a baseline activity of < 1 impulse/s and can be activated by lung inflation to 3-4 V_T (5). Next, the effects of capsaicin (0.2-10 μg/kg) injected into the right atrium were compared with those of the same dose of capsaicin injected into the left atrium. Pulmonary and bronchial C-fibers were distinguished by comparison of the latency of the response to capsaicin injection (5). If a fiber responded to right atrial injection of capsaicin within 1-3 s, but did not respond to left atrial injection, then it was considered as a pulmonary C-fiber. If a fiber responded to right atrial injection of capsaicin took 5-12 s, and the fiber also responded to left atrial injection, then it was classified as a bronchial C-fiber. At the beginning of each experiment, the nerve ending to be studied

was located by gentle palpation of the lung. Endings that could not be located within the lung were not studied.

Vagal Cooling

The procedures for vagal cooling have been described in detail elsewhere (17, 19). In brief, a segment (5-7 cm long) of cervical vagosympathetic nerve trunk was carefully separated from a carotid artery. The segment of nerve was placed in a groove (1 cm long) of a copper tube and covered with agar (4% in saline). Care was taken to avoid causing any tension on the nerve. A thermocouple was glued to the copper tube for measuring the temperature. The coolant (ethylene glycol) was pumped through a copper tube and kept at a constant preset temperature. The temperature was continuously recorded on a polygraph. Conduction velocities were measured by stimulating the cervical vagus nerve near its outlet from the thoracic cavity, through two pairs of stimulating electrodes, 2.3 cm apart, fixed in a shielded assembly.

Perivagal Capsaicin Treatment

The procedures for perivagal capsaicin treatment have been described in detail elsewhere (12, 17, 20). In brief, with the aid of a microscope, both cervical vagus nerves were desheathed over a 5 mm long segment, distal to where they were cooled. Strips of cotton soaked in capsaicin solution (2 to 10 mg/ml) were wrapped around the desheathed portion of the nerve for less than 30 min and then removed. Capsaicin (Sigma) was dissolved in 2.5% ethanol, 10% Tween 80, and 87.5% isotonic saline to make different concentrations of solution (2, 3, 6, and 10 mg/ml) for perivagal capsaicin treatment. The solution (2 mg/ml) was further diluted to 100 mg/ml by 0.9 % isotonic saline for right atrial injection of capsaicin.

Experimental Protocols

The effects of vagal cooling and of capsaicin on vagal afferent activity were studied in 29 dogs. Experiments were also conducted to find out if there were differences among the vagal afferent C-fibers in their sensitivity to perivagal capsaicin and to vagal cooling. First, the threshold of each fiber and the effects of vagal cooling on vagal afferent activity were measured. After the nerve was rewarmed, conduction velocity was measured. The effects of perivagal capsaicin were then studied. In some cases, various concentrations of capsaicin were applied to the nerves in a random order. In other cases, they were applied in sequence from low to high

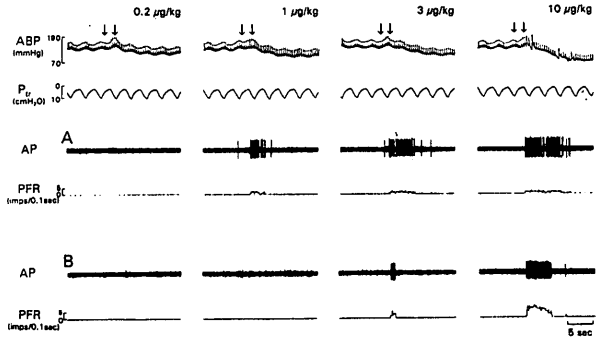

Fig. 1. Effects of different doses of vascular capsaicin on afferent activity of two lung vagal C-fibers. The first arrow indicates loading of capsaicin into catheter and the second indicates injection into right atrium. From top to bottom: ABP, arterial blood pressure; Ptr, tracheal pressure; AP, action potentials; PFR, peak firing rate. Note that activity of receptor in panel A was evoked beginning with a dose of 1 μg/kg, while that of receptor in panel B was evoked by 3 and 10 μg/kg.

concentrations (the first dose used usually was 2 mg/ml). If a dose did not block the afferent impulse activity after 30 min, the cotton was removed, and the nerve was rinsed and covered with warm paraffin oil. At least a 20 min recovery time elapsed before we applied a higher concentration of capsaicin and repeated the same procedure until the effective concentration was found. Each study consisted of a 2-min control recording period followed by a 30-min test period. During the test period, the data were recorded continuously. We usually recorded data of each dog from one pair of fibers using two electrodes simultaneously throughout all the tests.

Data Analysis and Statistics

Neural activity of lung vagal C-fibers and mean arterial blood pressure were measured in 1-s intervals. These physiological parameters were analyzed using a computer equipped with an analog/digital convertor (Gould DASA 4600) and software (BioCybernatics, 1.0). Results obtained from the computer analysis were routinely checked with those obtained by manual calculation for accuracy. Results were compared with a repeated measures one-way analysis of variance (ANOVA) or an unpaired *t*-test. If the ANOVA indicated a significant effect, the data were further analyzed with the Bonferroni method. $P < 0.05$ was considered significant. All data are presented as mean ± SE.

Results

Effects of Different Doses of Vascular Capsaicin

Two groups of lung vagal C-fibers were

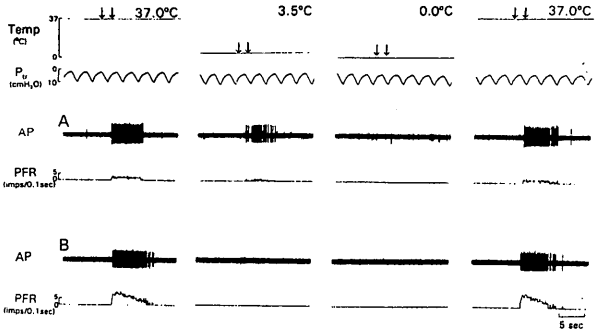

Fig. 2. Effects of vagal cooling on afferent activity evoked by right atrial injections of capsaicin in two lung vagal C-fibers. The first arrow indicates loading of capsaicin (3 μg/kg) into catheter and the second indicates injection into right atrium. From top to bottom: Temp, temperature; P_{tr}, tracheal pressure; AP, action potentials; PFR, peak firing rate. Note that the neural conduction of receptor in panel A was blocked at 0 °C, while that of receptor in panel B was blocked at 3.5 °C. Both C-fibers recovered when the temperature was returned to body temperature.

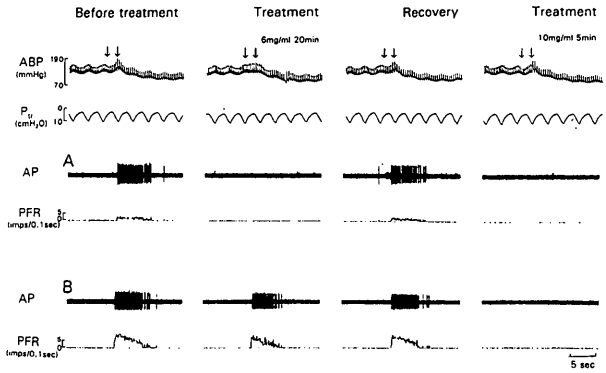

Fig. 3. Effects of perivagal capsaicin treatment on afferent activity evoked by right atrial injections of capsaicin in two lung vagal C-fibers. The first arrow indicates loading of capsaicin (1 μg/kg in panel A and 3 μg/kg in panel B) into catheter and the second indicates injection into right atrium. From top to bottom: ABP, arterial blood pressure; P_{tr}, tracheal pressure; AP, action potentials; PFR, peak firing rate. Note that a dose (6 mg/ml) that was three times as high as the threshold dose was applied to the nerve for 20 min. This dose had no significant effect on the conduction of receptor in panel B, but it blocked that of receptor in panel A. After receptor in panel A recovered to baseline activity, the conduction of both receptors was blocked after treatment with a higher concentration of capsaicin (10 mg/ml) for 5 min.

classified according to their sensitivities to different doses of capsaicin injected into the right atrium. Two representative examples are shown in Figure 1. As shown, the threshold dose for the receptor in panel A was 1 μg/kg, whereas that for the receptor in panel B was 3 μg/kg. Of the 89 C-fibers studied, 73 were similar to the receptor in panel A and had a mean threshold dose of 1.47 ± 0.31 μg/kg. The other 16 receptors were similar to the receptor in panel B and had a mean threshold dose of 3.45 ± 0.55 μg/kg.

Effects of Vagal Cooling

Two groups of lung vagal C-fibers were distinguished by their susceptibility to vagal cooling. Two typical examples are shown in Figure 2. As shown, at 3.5 °C, the activity of the receptor in panel B was blocked, but the receptor in panel A still responded to injection of capsaicin (10 μg/kg) into the right atrium. The receptor in panel A was finally blocked at 0 °C. Of the 41 capsaicin-sensitive fibers studied, 30 were similar to the receptor in panel A (Fig. 2) and had a mean blocking temperature of -0.4 ± 0.2 °C. The other 11 receptors were similar to the receptor in panel B (Fig. 2) and had a mean blocking temperature of 4.3 ± 0.3 °C.

Effects of Perivagal Capsaicin

Local application of lower doses of capsaicin (2, 3, 6 mg/ml) on cervical vagus nerve blocked the activity of some lung vagal C-fibers, but others were not blocked even when they were exposed to more than 10 mg/ml of the drug. Two typical receptors are shown in Figure 3. As shown, perivagal capsaicin treatment at a concentration of 6 mg/ml for 20 min blocked the conduction of the receptor in panel A, while it had no obvious effect on the receptor in panel B. However, a higher concentration of capsaicin (10 mg/ml, 5 min) blocked both receptors (Fig. 3, most far right tracings). Of the 89 lung vagal C-fibers studied, 73 were similar to the receptor in panel A (Fig. 3); most of them (52 of 73) were blocked by perivagal capsaicin with a concentration of less than 3 mg/ml (Fig. 4, lower left). The remaining 16 fibers were similar to the receptor in panel B (Fig. 3); their activities were not blocked by repeated application of capsaicin from 2 to 6 mg/ml. Furthermore, most (10 out of 16) of their conduction could be blocked by capsaicin when the concentration was greater than 10 mg/ml. The remaining 6 fibers, however, were not affected even at this high dose (Fig. 4, lower right).

Relationship between Effects of Vagal Cooling and Perivagal Capsaicin

The responses of lung vagal C-fibers to perivagal capsaicin and to vagal cooling are compositely shown in Fig. 4. The C-fibers were mapped based on their responses to capsaicin and to vagal cooling. As shown, the activity of the C-fibers with low resistance to perivagal capsaicin (LRC) was also blocked at relatively low temperatures (Fig. 4, upper left) ($p < 0.05$), while the activity of the fibers with high resistance to capsaicin (HRC) was blocked at relatively high temperatures (Fig. 4, upper right) ($p < 0.05$). Among

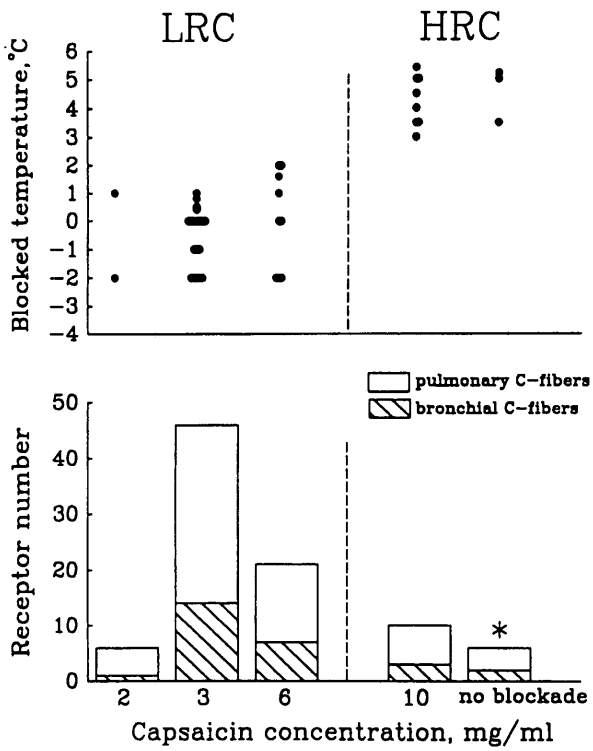

Fig. 4. Composite figures showing the relationship between effectiveness of blocking effects of perivagal capsaicin and cooling temperature on lung vagal C-fibers (upper panels; a single dot represents one fiber), and between concentrations of perivagal capsaicin and the numbers of C-fibers (lower panels). Note that afferent activity of LRC (low resistance to capsaicin) vagal C-fibers was blocked at relatively low doses of perivagal capsaicin and also at a relatively low temperature (upper left panel). Afferent activity of HRC (high resistance to capsaicin) vagal C-fibers was blocked at higher dose of perivagal capsaicin or not blocked at all, and they were also blocked at higher temperatures (upper right panel). *, receptor activity not blocked by capsaicin.

the 73 LRC fibers (Fig. 4, lower left), 51 were pulmonary C-fibers and 22 were bronchial C-fibers. Among the 16 HRC fibers (lower right), 11 were pulmonary C-fibers and 5 were bronchial C-fibers. In a total of 44 LRC C-fibers measured, the mean conduction velocity was 1.18 ± 0.15 m/s (n = 33, range 0.52 to 2.50 m/s). In a total of 11 HRC C-fibers measured, the mean conduction velocity was 1.33 ± 0.77 m/s (n = 11, range 0.66 to 2.60 m/s). There was no significant difference ($p > 0.05$) in the mean conduction velocity between LRC and HRC fibers.

Discussion

The results of this study clearly showed that lung vagal C-fibers could be grouped into two categories, LRC and HRC, based on their responses to perivagal capsaicin and to vagal cooling. Compared to HRC lung vagal C-fibers, the activity of LRC vagal afferents were blocked at relatively low concentrations of perivagal capsaicin and at relatively low temperatures. Thus, these observations support the hypothesis that there are two subgroups of lung vagal C-fiber afferents in dogs. The current classification is that lung vagal C-fiber afferents can be divided into two subpopulations: bronchial C-fibers and pulmonary C-fibers (5). This classification is based upon the fact that chemical stimuli can activate these two types of C-fibers through different circulatory assesibilities. Bronchial C-fibers are excited after a long delay by right atrial injection of capsaicin and after a shorter delay by injection into the left atrium (5). In this contest, pulmonary C-fibers are stimulated after a very short delay by right atrial injection of capsaicin and have no response to injection into the left atrium (5). The present findings showed no relationship between our and the current classifications. Our classification of LRC and HRC C-fibers also does not seem to correlate with the conduction velocities of the unmyelinated fibers conveying the activity of these two groups of C-fibers.

The mechanism responsible for the differences among lung vagal C-fibers in their response to vascular or perivagal capsaicin is not fully understood. This group of fibers may have different membrane receptors (7) or different membrane properties (23), and thus some fibers in the dorsal roots may be selectively vulnerable to capsaicin (22). Capsaicin evokes an inward current which leads to membrane depolarization (21, 24). This depolarization blocks the conductance of afferent C-fibers (7, 21, 23, 24) and inhibits axoplasmic transport (9). Differences in the distribution or density of receptors could account for the present finding. Differences in the relative sensitivity of receptors to capsaicin may also be responsible. Petsche et al. (23) has reported that when capsaicin was applied to axons of rat coccygeal or saphenous nerves, nociceptive C-fibers that responded to strong mechanical stimuli and to heat were blocked, while those that responded to cold were not affected. Their data indicate that differences between these fibers exist both in the nerve endings and the axons.

One possible cause for the difference in the susceptibility of lung vagal C-fiber to perivagal capsaicin may arise from the different diffusion time of capsaicin to the fiber recorded. Since all the receptors whose activity was recorded were picked at random, and the same concentrations of capsaicin were applied each time, it is unlikely that this is the case. Furthermore, Craft and Porreca (6) reported that the most important factor in the desensitization of sensory afferent fibers caused by topical capsaicin is the dose, and there appears to have no diffusion barrier to capsaicin (23).

It has been shown that the injection rate (25) and

dose (4) of capsaicin may determine the type of vagal C-fibers being stimulated. For instance, if capsaicin is slowly injected into the pulmonary artery, there is rapid shallow breathing but no apnea (5, 25). It is possible that this response is due to stimulation of the LRC lung vagal C-fibers defined in this study. Conversely, when a bolus of capsaicin is rapidly injected, an apnea ensues (25). This response may possibly be due to a simultaneous stimulation of both HRC and LRC lung vagal C-fibers. The present distinction between LRC and HRC fibers is consistent with those data. Based upon the present findings, the population in the group of HRC lung vagal C-fibers is apparently smaller than that of LRC lung vagal C-fibers. However, the relative contributions of these two groups of receptors to the C-fiber-mediated respiratory reflexes are not known at the present. It is unlikely that the possible tissue damage caused by perivagal capsaicin treatment and vagal cooling may contribute to the difference in the population size of these two fiber groups. This is because, in a parallel study, we found that C-fiber responses to lung hyperinflation and to capsaicin injection could reappear after having been blocked by these two experimental interventions, providing that recovery time was long enough.

A study in dogs (13) reported that most of the hyperinflation-evoked increases in the activity of slowly and rapidly adapting receptors (two types of vagal myelinated afferents) are blocked at temperatures (6-7 °C) higher than those required to block the unmyelinated afferent C-fibers (about 0 °C). Hargreaves et al. (11) reported that the increase in respiratory rate evoked by stimulation of rapidly adapting receptors is attenuated when cervical vagi were cooled to 8-9 °C to remove the influence of lung myelinated vagal afferents. In the present study, when the temperature was further lowered to 4.5 °C to ensure complete blockade of the myelinated fibers, the activity of LRC fibers still continued (Figs. 2 and 4). It appears that vagal cooling is not a technique producing a clear-cut blocking effect on C-fiber activity.

It is known that a variety of chemical stimuli can stimulate both lung vagal C-fiber afferents and rapidly adapting receptors (2, 3, 5, 15-17). Additionally, these two types of pulmonary receptors play an important role in eliciting similar airway responses such as bronchoconstriction (14), increase in airway secretion (26, 30), and changes in breathing patterns (5, 29). Therefore, it is important to differentiate the contribution originating from these two types of pulmonary receptors. For this reason, vagal cooling and perivagal capsaicin treatment have been widely used as techniques to explore the specific functions of lung vagal C-fiber afferents and they have been justified to selectively or preferentially block the respiratory reflexes originated from C-fiber afferents (5, 17, 19, 20). Accordingly, the results obtained from this study may provide information for researchers to further differentiate the respiratory reflexes originating from these two subgroups, LRC and HRC, lung vagal C-fibers.

In summary, our data show that lung vagal C-fiber afferents can be grouped into two categories, LRC and HRC, based on their responses to perivagal capsaicin treatment and to vagal cooling.

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