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Right and left vagus nerves regulate breathing by multiplicative interaction

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ABSTRACT

Although it has been recognized for more than a century, we still do not know how the two vagus nerves interact to produce Hering–Breuer reflex. In the current study, we tested the hypothesis that the vagus nerves interact via a multiplicative effect. We examined the Hering–Breuer reflex before and after unilateral (first) and then bilateral (second) vagotomies in the mouse. The lung is mostly innervated homolaterally. Since the right and left lung formed 68.2 and 31.8% of total lung weight, if the interaction is mediated by an additive mechanism, unilateral vagotomy would remove the reflex effects by 68.2 and 31.8%, respectively. Instead, unilateral vagotomy removed 85.4 ± 6.0% (>68.2%) or 52.8 ± 3.7% (>31.8%) of the reflex effects on respiratory rate ($n = 9$, $P < 0.05$); and removed 79.1 ± 4.5% (>68.2%) or 59.3 ± 9.1% (>31.8%) of the effect on expiratory pause induced by lung inflation ($n = 12$, $P < 0.05$). Since the first vagotomy removes more reflex effect than the second vagotomy, we conclude that the two vagus nerves exert their Hering–Breuer reflex effects by a multiplicative effect.

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1. Introduction

The research about the effect of the vagus nerve on respiration can be traced back to 19th century. In 1868, Breuer proposed a concept of “self-steering of breathing by the vagus nerves” (Breuer, 1868). Vagotomy caused a much slower and deeper breathing pattern. In 1933, Adrian explained the underlying mechanism and stated that this feedback control is due to phasic increase in discharge from pulmonary stretch receptors during inspiration (Adrian, 1933; Bartoli et al., 1973). The vagus nerve includes afferent and efferent fibers (Lee and Yu, 2014). The former conveys mechanical and chemical information from the lung to the respiratory center to reflexively regulate respiratory activity. The lung is mostly innervated by the ipsilateral vagus nerve. However, regulatory effects generated from the two lungs are synchronized. Thus, there should be an efficient interaction between the two vagus nerves. Though the synergistic interaction between subtypes of airway vagal afferent nerve has been reported (Mazzone et al., 2005), little is known about the right and left vagus nerve interactions. In this study, we hypothesize that the two vagus nerves act by a multiplicative effect. To test this hypothesis, we analyzed the Hering–Breuer reflex before and after unilateral and bilateral vagotomy. In mice, the right lung is twice as big as the left lung. If the two nerves interact additively, unilateral vagotomy should remove 2/3 (right) and 1/3 (left) of the reflex effects; however, if multiplicatively, it should remove significantly greater reflex effects.

2. Materials and methods

2.1. Animal

Adult C57/B6 male or female mice (6–8 week-old) were fed standard rodent chow and housed in a disease-free barrier facility with 12 h light/dark cycles. The study conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-53). The Institutional Animal Care and Use Committee at University of Louisville and the Robley Rex VA Medical Center approved the use of animals and the study protocol.

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2.2. Vagotomy

After overnight fasting, mice were anesthetized with a mixture of ketamine (87 $\mu g\,g^{-1}$) and xylazine (13 $\mu g\,g^{-1}$) intraperitoneally. The neck was opened with a midline incision and the trachea was cannulated. Left and right vagus nerves were identified under a binocular microscope at 10× magnification. A minimum of 5 mm of one of the nerves was resected. Since the aortic and sympathetic nerve trunks are not separated from the vagus nerve in the mouse, these nerves were sectioned along with the vagus nerve. Right or left vagotomy was performed on 50% of mice for initial testing, and then a second vagotomy was performed on the opposite side. Therefore, effects of right and left vagus nerves could be compared.

2.3. Assessment of Hering–Breuer reflex

In anesthetized mice, Hering–Breuer reflexes were examined before and after unilateral and then bilateral vagotomies. Respiratory variables were measured with a Buxco FinePointe RC system (Buxco Electronics, Inc., USA). This system includes a mouse respirator, a chamber (562 cm^3), and flow and pressure transducers. Spontaneously breathing mice were placed in the chamber (as a plethysmograph) to measure respiratory rate for one minute. For measuring expiratory pause during lung inflation, mice were mechanically ventilated at 150 cycles/min with 0.2 ml through the tracheal cannula, which was connected to a transducer to measure airway pressure. Respiratory variables were measured at a steady state. Two minutes were allowed after initiation of mechanical ventilation and after each measurement the animals were allowed to resume spontaneous breathing. At least 10 min elapsed between measurements (and between vagotomy and measurement) to allow for recovery. The resulting data were analyzed with a data acquisition system (iworx LabScrib2, Dover, NH). A positive airway pressure of 20 or 30 cmH_2O (generated by an air supply system with adjustable pressure) was applied for assessing the Hering–Breuer reflex, during which the animal was switched from mechanical ventilation to the targeted pressure via a three way stopcock. To quantify the Hering–Breuer reflex, expiratory pause was determined by measuring time from the application of pressure to the onset of inspiration during lung inflation (Merazzi and Mortola, 1999). Bilateral vagotomy was confirmed by the disappearance of the Hering–Breuer reflex at an inflation pressure of 30 cmH_2O.

2.4. Statistical analysis

Data are presented as group mean ± SE. Differences between two groups of data from the same animals were evaluated by the two-tailed paired *t*-test. Comparisons between groups with different animals were performed by ANOVA followed by the Tukey's post hoc test for three groups and an independent *t* test for two groups. $P < 0.05$ was considered significant.

3. Results

3.1. Lung weight

Right and left lungs from 36 mice were weighed separately. The right lung (101 ± 7 mg) weighed significantly more than the left lung (46 ± 3 mg, $P < 0.001$), accounting for 68.2 ± 1.7% and 31.8 ± 1.7% of the total weight, respectively.

3.2. Effect of vagotomy on respiratory rate

In 18 spontaneously breathing mice, consecutive unilateral followed by bilateral vagotomies decreased the respiratory rate (RR) from 232.7 ± 18.8 to 134.8 ± 15.7 and to 110.8 ± 15.7 breaths per minute (bpm) when vagotomy was performed on the right nerve first; and from 242.5 ± 21.9 to 185.5 ± 17.2 and to 137.2 ± 19.2 bpm when vagotomy was performed on the left nerve first (Fig. 1 and Fig. 2A). The respiratory rate is similar after bilateral vagotomy in right vagotomy first and left vagotomy first groups (110.8 ± 15.7 vs 137.2 ± 19.2, $n = 9$, $P = 0.307$). To determine whether the right and left vagus nerves interact additively or multiplicatively, we examined the percentage effects of unilateral vagotomy, using the following formulation $\Delta RR\% = (RR_{UV} - RR_{VI})/(RR_{BV} - RR_{VI}) \times 100$ (RR: respiratory rate; VI: vagal intact; UV: unilateral vagotomy; BV: bilateral vagotomy). We found that right and left vagotomy removed more reflex effects relative to lung size [85.4 ± 6.0% (>68.2%) and 52.8 ± 3.7% (>31.8%) (both $n = 9$, $P < 0.05$)]. Interestingly, unilateral vagotomy removed more reflex effect than it did after the contralateral vagus nerve was sectioned. For example, right vagotomy removed 85.4 ± 6.0 and 47.2 ± 3.7% ($n = 9$, $P < 0.001$); left vagotomy removed 52.8 ± 3.7 and 14.6 ± 6.0% of the reflex effect ($n = 9$, $P < 0.01$) (Fig. 2B). Average effect of right vagotomy (first vagotomy and second vagotomy) was greater than that of left vagotomy (66.3 ± 6.3 vs 33.7 ± 6.3%, $n = 18$, $P < 0.001$) (Fig. 2C). The averaged effect of the first vagotomy (69.1 ± 5.8%) was nearly twice that (30.9 ± 5.8%) of the second vagotomy ($n = 18$, $P < 0.001$) (Fig. 2D), supporting the theory of a multiplicative interaction between the two vagus nerves.

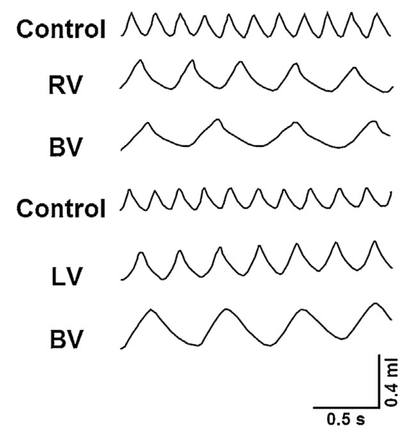

Fig. 1. Effects of vagotomy on breathing pattern. Representative recordings show that unilateral (left or right) vagotomy decreased respiratory rate; bilateral vagotomy decreased respiratory rate further. The effect of RV was greater than that of LV. RV: right vagotomy; LV: left vagotomy; BV: bilateral vagotomy. *Y*-axis is lung volume and *X*-axis is time.

3.3. Effect of vagotomy on expiratory pause induced by lung inflation

Twenty four mechanically ventilated mice were assessed for Hering–Breuer reflex in response to lung inflation with a 30 cm H_2O constant pressure. Unilateral vagotomy decreased expiratory pause induced by lung inflation. After bilateral vagotomy, the Hering–Breuer reflex was completely abolished (Fig. 3). Twelve mice received 2 levels of lung inflation (20 and 30 cm H_2O, $n = 6$ in each group). Expiratory pause was significantly shorter in right vagotomized mice (1.71 ± 0.52 s and 4.26 ± 1.74 s at 20 and 30 cm H_2O) than in left vagotomized mice (3.74 ± 1.90 s and 10.07 ± 4.40 s) (Fig. 4A). Unilateral vagotomy removed more reflex effect than it did after the contralateral vagus nerve was sectioned. For example, right (first and second) vagotomies removed 79.1 ± 4.5 and 40.7 ± 9.1% ($n = 12$, $P < 0.05$) of the reflex effect, whereas, left (first and second) vagotomies removed 59.3 ± 9.1 and 20.8 ± 4.5% ($n = 12$, $P < 0.05$), respectively (Fig. 4B). The right vago-