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Characterization of thoracic spinal neurons with noxious convergent inputs from heart and lower airways in rats

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Abstract

Respiratory symptoms experienced in some patients with cardiac diseases may be due to convergence of noxious cardiac and pulmonary inputs onto neurons of the central nervous system. For example, convergence of cardiac and respiratory inputs onto single solitary tract neurons may be in part responsible for integration of regulatory and defensive reflex control. However, it is unknown whether inputs from the lungs and heart converge onto single neurons of the spinal cord. The present aim was to characterize upper thoracic spinal neurons responding to both noxious stimuli of the heart and lungs in rats. Extracellular potentials of single thoracic (T3) spinal neurons were recorded in pentobarbital anesthetized, paralyzed, and ventilated male rats. A catheter was placed in the pericardial sac to administer bradykinin (BK, 10 µg/ml, 0.2 ml, 1 min) as a noxious cardiac stimulus. The lung irritant, ammonia, obtained as vapor over a 30% solution of NH₄OH was injected into the inspiratory line of the ventilator (0.5–1.0 ml over 20 sec). Intrapericardial bradykinin (IB) altered activity of 58/65 (89%) spinal neurons that responded to inhaled ammonia (IA). Among those cardiopulmonary convergent neurons, 81% (47/58) were excited by both IA and IB, and the remainder had complex response patterns. Bilateral cervical vagotomy revealed that vagal afferents modulated but did not eliminate responses of individual spinal neurons to IB and IA. The convergence of pulmonary and cardiac nociceptive signaling in the spinal cord may be relevant to situations where a disease process in one organ influences the behavior of the other.

Keywords

Visceral nociception; cardiopulmonary reflex; sympathetic afferent; vagal afferent; spinal cord

Introduction

Noxious stimulation of heart and/or lungs evokes profound alterations in autonomic cardiopulmonary activity via peripheral and central neural pathways. For example, a characteristic cardiopulmonary reflex (hyperpnea, tachypnea and hypotension) is elicited by stimulation of pulmonary sympathetic afferents with intrapulmonary bradykinin (Soukhova et al. 2003; Wang et al. 2003). Dramatic responses in the cardiovascular and respiratory systems to noxious stimuli of heart and lungs also may relate to some pathological conditions. For example, dyspnea is an important symptom among patients with suspected and known coronary artery disease and a significant predictor of the risk of death from cardiac causes (Abidov et

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al. 2005). It is reasonable to assume that the convergence and interaction of nociceptive afferent inputs from heart and lungs to the same central neural pathway or circuit could be involved in cardiorespiratory pathological reflexes and conditions. Indeed, it has been demonstrated that afferent inputs of pulmonary C-fibers and cardiac receptors converge onto single neurons in the nucleus of the solitary tract (NTS) in cats and mouse. These cardiopulmonary convergent neurons are thought to be involved the defensive or protective cardiopulmonary reflexes (Paton 1998) as well as some pathological states such as pulmonary congestion and cardiac failure (Silva-Carvalho et al. 1998).

The spinal cord is another important station for integrating primary afferent inputs from heart and lungs. For example, cardiac nociceptive information associated with angina pectoris and nociceptive reflexes are transmitted by sympathetic afferent A-delta and C fibers. These fibers enter the upper thoracic spinal cord and synapse on cells of origin of the spinothalamic tract, spinoreticular tract and other ascending pathways that transmit information to the thalamus, medullary reticular nuclei and other areas of the brain (Foreman 1999; Meller and Gebhart 1992). However, there are few available data that explore intraspinal processing and integration of noxious pulmonary afferent information. Recently, our laboratory and another group reported that noxious information resulting from inhaled ammonia that reached the lower airways is transmitted to the spinal cord and influences activity of upper thoracic spinal neurons (Hummel et al. 1997; Qin et al. 2007). The aim of the present study in rats was to examine whether noxious afferent inputs from heart and lungs converge onto single spinal neurons in the upper thoracic spinal cord. Several of the spinal neurons with convergent inputs from heart and lungs used in this study were also used for characterization of pulmonary input in another study by Qin et al. (2007). A preliminary report of this work has been published in an abstract (Foreman et al. 2005).

Results

Of 65 upper thoracic (T3) spinal neurons responding to inhaled ammonia (IA), 58 neurons (89%) also responded to intrapericardial bradykinin (IB). Forty-nine cardiopulmonary convergent neurons were recorded from left side and 9 neurons from right side of the spinal cord. Fourteen cardiopulmonary convergent neurons were located in superficial laminae of dorsal horn (depth <0.3 mm) and 44 neurons were found in deeper laminae (depth 0.3–1.2 mm). A comparison of proportions of superficial and deeper neurons responding to visceral stimuli is shown in Fig. 1A, B. As a control for non-noxious pulmonary stimulation, inhaled room air did not affect spinal neuronal activity (6.9 ± 2.5 vs 7.4 ± 2.1 imp/s), but IA significantly increased activity from 7.1 ± 2.7 imp/s to 30.9 ± 4.7 imp/s ($P < 0.01$, $n = 6$). Similarly, intrapericardial normal saline (a vehicle for dissolving bradykinin) did not affect spinal neuronal activity (7.6 ± 2.2 vs 7.8 ± 2.6 imp/s), but IB significantly increased activity from 6.7 ± 2.8 imp/s to 22.3 ± 4.8 imp/s ($P < 0.01$, $n = 8$). Electrolytic lesions of recording sites for spinal neurons responding to both IA and IB were verified histologically. Neurons excited by both IA and IB were primarily located in laminae I, II, III, V and VII, whereas the majority of spinal neurons with complex response patterns (see next paragraph) to IA and/or IB were found in laminae V and VII (Fig. 1C–E).

Multiple patterns of convergent input from lungs and heart onto spinal neurons were observed. A majority (47/58, 81%) of viscerovisceral neurons were excited (E) by both IA and IB and classified as E/E neurons; 2 neurons were inhibited (I) by both stimuli and classified as I/I neurons; whereas the remainder exhibited complex response patterns, including 6 I/E, 2 E-I/E, and 1 E/E-I neurons (E-I, an excitatory response was followed by inhibition). Examples of cardiopulmonary convergent neurons with different response patterns to IA and IB are shown in Fig. 2A–C. A summary of the characteristics of excitatory responses to IA and IB is shown in Table 1. Furthermore, spinal neurons excited by IA were subdivided into two groups on the

basis of the recovery time of responses to control level of activity (Qin et al. 2007): neurons with recovery time ≤ 30 seconds were classified as short-lasting ($n=24$) responses, and neurons with recovery time > 30 seconds were classified as long-lasting ($n=23$) responses. Similar to classification of responses to IA, spinal neurons excited by IB also were divided into two groups based on the recovery time of responses to control level of activity (Qin et al. 2003): 25 neurons with short-lasting responses to IB (≤ 50 s) and 22 neurons with long-lasting responses to IB (> 50 s). A comparison of short- and long-lasting excitatory response patterns of viscerovisceral convergent neurons is as follows: 16 neurons had short-lasting responses and 14 neurons had long-lasting responses to both IA and IB; 7 neurons respectively had short- and long-lasting responses to IA and IB; 10 neurons respectively had long- and short-lasting responses to IA and IB. Additionally, of 13 spinal neurons responding to both IA and IB, 5 (39%) neurons also responded to hyperinflation of lungs. These hyperinflation-responsive neurons were excited by IB, but exhibited different responses to IA (2 E, 1 E-I, 1 I-E, and 1 I).

Eight spinal neurons responding to both IA and IB were recorded before and after bilateral cervical vagotomy in 8 animals. Vagotomy had variable effects on excitatory responses of individual cardiopulmonary convergent neurons to IA or IB (Table 2, Fig. 3). However, the average excitatory responses of cardiopulmonary convergent neurons to IA or IB ($n=8$) were not different from those before vagotomy (Table 3). Spinal neuronal responses to IA and IB were evaluated after intrapericardial lidocaine and compared to control responses before lidocaine (as % of control). Individual neuronal responses to IA and IB for each animal are shown at different time intervals after lidocaine was placed in the pericardial sac (Fig. 4). While lidocaine reduced the response to ammonia in 2 of the 3 examples, effects on the bradykinin response were considerably greater. Effects of lidocaine on arterial pressure were minimal.

Discussion

Responses to cardiac and pulmonary stimuli

Activation of the thoracic spinothalamic tract, spinoreticular tract and spinal neurons is produced by electrical stimulation of cardiopulmonary sympathetic nerve in monkeys, cats and rats (Foreman 1999). However, the question of convergence of pulmonary and cardiac inputs onto single spinal neurons has not been considered. In the present study, inhaled ammonia and intrapericardial bradykinin were used to activate pulmonary and cardiac nociceptors, respectively. Ammonia is a strong irritant of the airway and lungs, which can cause symptoms such as burning of the eyes, nose, throat and uncomfortable sensations (Morton et al. 1950;Tepper et al. 1985). Inhaled ammonia in animals has been shown to activate vagal pulmonary A-delta and C afferents or receptors (Kohl and Koller 1980;Matsumoto 1989;Wang et al. 1996) as well as upper thoracic spinal neurons (Hummel et al. 1997;Qin et al. 2007). Intrapericardial bradykinin was used as a noxious cardiac stimulus for activating vagal and sympathetic cardiac receptors because it can be released during myocardial ischemia (Hashimoto et al. 1977;Kimura et al. 1973). Stimulation of these receptors can elicit angina pectoris and autonomic responses (Gaspardone et al. 1999).

In the present study, 58/65 upper thoracic (T3) spinal neurons (89%) responding to IA also responded to IB. All of the superficial cardiopulmonary convergent neurons ($n=13$) had excitatory responses to both IA and IB, whereas deeper spinal neurons sometimes exhibited complex viscerovisceral convergent patterns of responses (33 E/E; 11 multiple patterns). The proportion of cardiopulmonary convergent spinal neurons is different from a previous observation examining cardiorespiratory convergence in the NTS, another integration station of primary cardiac and pulmonary afferent inputs. Those studies showed that 46% of the cardioceptive NTS neurons in the cat and 71% of the neurons in the mouse received input from the heart and pulmonary C vagal fibers (Silva-Carvalho et al. 1998;Paton 1998). An important difference between their and our studies is that the convergence resulted from

activation of vagal afferent input in the previous studies but spinal sympathetic afferent fibers in the present study. This may account for the variation in the number of neurons that received convergent inputs from heart and lungs. The use of different animal species also may account for the different results.

Based on the duration of the excitatory responses, the present study identified short- and long-lasting groups of spinal neuronal responses to both noxious pulmonary or cardiac inputs. Previous studies suggest that the short-lasting responses are associated with A-delta afferent input and long-lasting responses with C fibers afferent activity (Qin et al. 2003;2007). In the present study, 64% (30/47) of cardiopulmonary spinal neurons had either short- or long-lasting excitatory responses to both IA and IB, which suggested that these neurons received cardiopulmonary afferent inputs traveling in the same A-delta or C-fibers. In contrast, 36% (10/47) neurons had short- and long-lasting excitatory responses with different effects from IA and IB, which suggested these spinal neurons received cardiopulmonary convergent inputs from different afferent fibers.

Spinal neurons observed in the present study might include second-order sensory neurons projecting to supraspinal nuclei and other spinal segments. Furthermore, recordings from interneurons in the spinal cord that transmit afferent information to ventral horn motor neurons also might be included. Convergence of heart and lung inputs might occur by one or more mechanisms. It has been shown that 6–11% of the dorsal root ganglion (DRG) neurons have axons that innervate both the colon and urinary bladder at the lumbosacral and thoracolumbar spinal cord in rats (Christianson et al. 2006). However, dual innervation from heart and lungs has not been examined, although 7–14% of the DRG neurons were double-labeled following injections of dyes into pericardial sac and upper arm somatic nerves (McNeill and Burden 1986; Alles and Dom 1985). Second, the afferent fibers from lungs and heart may synapse onto the same spinal neurons. This convergence would seem to be the most likely possibility because approximately 90% of spinal neurons responded to both IA and IB in the present study.

Differentiation of cardiac and pulmonary stimuli

Bradykinin in the pericardial sac and inhaled ammonia might possibly influence nociceptors in the lungs and heart, respectively, by distribution through the circulation. However, when nerve fibers in pericardial sac were blocked with lidocaine, there were much larger reductions in spinal neuronal responses to bradykinin than to ammonia (Fig. 4). Since lidocaine also has access to the circulation from pericardial sac, the observed reductions in ammonia response could have been produced by lidocaine reaching the lungs or even by effects at the spinal level. Low doses of lidocaine produce analgesia after intravenous administration (Ness 2000). We interpret the much greater and persistent effect on bradykinin responses as being due to the higher concentration of drug in the pericardial sac, and that bradykinin in the pericardial sac mainly acts on the heart. While bradykinin elicits a neural response from lung tissue with a small bolus injection, bradykinin released from the pericardial sac in the present experiment would likely be broken down by peptidases in the lungs (Dragovic et al. 1993). We also attempted to verify a lung specific effect using lung hyperinflation, but the results as to whether that stimulus only influenced the lungs were unclear.

Despite the circulatory distribution of lidocaine from pericardial sac, it could also be argued that ammonia delivered to the lungs by our protocol variably influenced nociceptors in the heart through the arterial circulation. Therefore, the reductions in ammonia response could be due to blockade of nerve fibers on the heart by lidocaine (Fig. 4). Against this argument is our preliminary finding that when ammonia inhalation influenced the activity of lumbosacral spinal neurons during bladder stimulation, the only way to eliminate the effect was to transect the spinal cord at the low thoracic segments (Farber et al. 2005). This result suggested that the effects of ammonia were through a neural pathway rather than by distribution to the bladder

via the arterial circulation. Also, for 2 of 3 neurons in Fig. 4, there was at least one post lidocaine time point where there was no depression of ammonia response while bradykinin response was substantially reduced. Our present experimental protocols do not allow us to exclude that some overflow of stimuli occur between lung and heart, but the differential effects of pericardial sac lidocaine on bradykinin and ammonia responses support the idea that these agents primarily act on heart and lungs, respectively.

Afferent pathway and vagal modulation

The upper thoracic spinal segment (T3) in the present study was selected for examining neuronal responses to noxious cardiopulmonary stimuli because this spinal segment receives primary sympathetic afferent fibers innervating heart (Hopkins and Armour 1989; Kuo et al. 1984) and lower airways or lungs (Dalsgaard and Lundberg 1984; Kostreva et al. 1975; Kummer et al. 1992; Plato et al. 2006). Previous studies have examined the effects of chemical stimulation of cardiac receptors on spinothalamic tract neurons and spinal neurons in the upper thoracic (T1–T5) spinal cord of primates, cats, and rats (Euchner-Wamser et al. 1994; Foreman 1999; Qin et al. 2003). Ammonia irritation of lower airways in rats activates upper thoracic (T2–T4) spinal neurons (Hummer et al. 1997; Qin et al. 2007). Immunohistochemical and electrophysiological analyses shows that approximately 70% of T1–T4 dorsal root ganglion neurons innervating lower airway are C fibers and the remainder are A-delta fibers. Some of these neurons contain substance P and also are sensitive to capsaicin stimulus in guinea-pigs and mouse (Dinh et al. 2004; Oh et al. 2006).

In the present study, bilateral cervical vagotomy did not eliminate the excitatory neuronal responses to either IA or IB. This result is consistent with observations in previous studies, in which thoracic spinal neuronal responses were examined for effects of intracardiac BK in cats with vagotomy (Bolser et al. 1989) and ammonia irritation in rats with vagotomy (Hummel et al. 1997; Qin et al. 2007). Therefore, it is suggested that the afferent effects on thoracic neurons of epicardial BK and IA are likely to result from the stimulation of cardiac and pulmonary sympathetic primary afferents. Although bilateral cervical vagotomy did not eliminate spinal neuronal responses to IA or IB, variable effects on the IA- or IB-evoked activity of individual spinal neurons were produced. This finding suggests that vagal afferent activity can exert modulatory influences on thoracic cardiopulmonary convergent spinal neurons with noxious inputs from heart and lungs. In support of this, previous studies show that electrical stimulation of vagal afferents suppress excitatory responses of thoracic spinothalamic tract neurons to activation of cardiopulmonary nerve in monkeys (Foreman 1999) and reduce c-fos expressing neurons in thoracic spinal cord during coronary artery occlusion and administration of intrapericardial algogenic chemicals in rats (Hua et al. 2004).

Possible physiological implication

The cardiopulmonary convergent spinal neurons responding to both noxious inputs from heart and lungs in the present study might contribute to central neural circuits of either cardiac- or pulmonary-induced visceromotor and autonomic reflexes. For example, injecting bradykinin, as inflammatory mediator, into the lung parenchyma after bilateral vagotomy in rabbits still evokes significant cardiovascular and respiratory responses, i.e. hyperpnea, tachypnea and hypotension (Soukhova et al. 2003; Wang et al. 2003). Irritation of the lungs or release of chemicals during myocardial ischemia in heart might activate spinal (sympathetic) afferents and excite thoracic cardiopulmonary convergent spinal neurons. The activation of these neurons might signal or even contribute to pathological “cross-talk” of the heart and lungs (Abidov et al. 2005).

Experimental Procedure

Experiments were performed in 34 male Sprague-Dawley rats (Charles River Inc.) weighing between 350–460 g. The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center and also followed the guidelines of the International Association for the Study of Pain. After initial anesthesia with sodium pentobarbital (60 mg/kg, i.p.), catheters were inserted into the right carotid artery to monitor blood pressure and into the left jugular vein to infuse sodium pentobarbital (15–25 mg/kg/hr) to maintain a continuous administration of anesthesia throughout the experiment. A tracheotomy was performed for artificial ventilation using a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). Animals were paralyzed with initial injection of pancuronium bromide (0.4 mg/kg, i.p.) followed by 0.2 mg/kg (i.p.) each hour during the experiment. A thermostatically controlled heating pad and overhead infrared lamps was used to keep rectal temperature between 37 and 38°C.

To irritate the lower airways, inhaled ammonia (IA) was used (Qin et al. 2006). Ammonia vapor from a 150 ml bottle filled containing 25–30 ml ammonia solution (28–30%) was drawn into a syringe. Volumes of 0.5 or 1.0 ml of this ammonia vapor were injected manually through the inspiratory tube of the ventilator over 20 seconds. This allowed the ammonia into lower airways after 7–10 breaths of 3–5 ml stroke volume of artificial ventilation. Multiple IA installations were examined for spinal neuronal responses at an interstimulus intervals of >8 min to avoid desensitization (Hummel et al. 1997). A noxious pulmonary mechanical stimulus, hyperinflation of the lungs, was produced by clamping the expiratory line of the ventilator to inflate the lungs with 3–4 breaths. In 6 experiments, a volume of 1.0 ml room air was injected into the inspiratory tube of ventilator over 20 seconds as control of non-noxious pulmonary stimulation. To activate cardiac receptors, a silicone tube (0.020 ID, 0.037 OD, 14–16 cm in length) with 5–8 small holes in distal 2 cm was carefully inserted into the pericardial sac over the left ventricle (Qin et al. 2003). The solution of bradykinin (10^{-5} M, 0.2 ml) was injected into the pericardial sac via a 1 ml syringe that was connected to the silicone tube. After one minute, bradykinin was removed and followed by 2–3 saline flushes (0.2 ml each) for rinsing the pericardial sac. In 8 experiments, warm physiological saline (0.2 ml) was injected into the pericardial sac for 1 min as control for effects of intrapericardial bradykinin on the spinal neurons. To examine whether ammonia inhalation (IA) likely affected pericardial receptors or whether intrapericardial bradykinin (IB) likely affected lung receptors, the nerve fibers and endings in pericardium and heart surface were blocked using intrapericardial lidocaine (2%, 0.2ml) for 10 min in three animals. Spinal neuronal responses to IA and IB were evaluated after intrapericardial lidocaine and compared to control levels before lidocaine (as % of control). In addition, the left and right cervical vagus nerves were separated from the carotid artery and silk suture was looped around each nerve trunk. Bilateral vagotomy was performed in some animals to interrupt visceral pathways.

Thoracic laminectomies were performed to expose T3 spinal segments for recording neuronal activity. After rats were mounted in a stereotaxic headholder, the dura mater of the T3 spinal segment was carefully removed and the spinal cord was covered with warm agar (3–4% in saline) to improve stability for neuronal recording. Carbon-filament glass microelectrodes were used to record extracellular action potentials of single spinal neurons. All recordings were made 0.5–2 mm lateral from the midline and at depths between 0–1.2 mm from dorsal surface of spinal cord. Signals were amplified and fed into a window discriminator. Discriminator output was displayed on an oscilloscope and recorded using a computer with Spike 3 data acquisition system (CED, Cambridge) to document neuronal activity. Extracellular potentials and simultaneous discharges from the window discriminator were monitored closely to make sure activity was recorded from only one unit. Configurations of action potentials and response characteristics of single units were the same throughout the experimental procedures. In a few

cases, the digital filter of Spike 3 program was used to select a single unit from multiple recordings of two spinal neurons for further analysis. A neuronal response (imp/s) to visceral stimulation was calculated by subtracting the mean of 10 s of control activity from the mean of 10 s of the greatest activity evoked by cardiac and pulmonary stimuli. A neuron was identified as responsive to various stimuli if the maximal change in activity was at least 20% compared to control activity (Bolser et al. 1991; Chandler et al. 1991; Hobbs et al. 1989). For neurons with no spontaneous activity, the minimum threshold of response was ≥ 2 imp/s (Hobbs et al. 1989). Data are presented as mean $\pm$ SE. Statistical comparisons were made using Student's paired or unpaired *t*-test and *Chi*-square test. Differences were considered statistically significant at $P < 0.05$.

To mark the locations of spinal neurons, electrolytic lesions (50 μ A DC, 20 s) were made at recording sites after neurons with visceral inputs had been studied. At the end of the experiment, animals were euthanized with an overdose of pentobarbital. The thoracic spinal cord was removed and placed in 10% buffered formalin solution. After at least three days, frozen sections (55–60 μ m) of the upper thoracic cord were made and lesion sites in the spinal cord were viewed under a microscope. Laminae of gray matter were identified using the description of the cytoarchitectonic by Molander et al. (1989).

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Fig 1.

Response patterns and recording sites of upper thoracic (T3) spinal neurons to inhaled ammonia (IA) and intrapericardial bradykinin (IB). *A, B*: Comparison of populations of superficial and deeper spinal neurons with cardiac and/or pulmonary inputs. R/NR, neurons with response (R) to IA and no response (NR) to IB. E/E, neurons with excitatory (E) responses to both IA and IB. MR/MR, neurons multiple response (MR) patterns to IA and IB (see text). *C-D*: Locations of spinal neurons responding to IA and/or IB. The black circles represent neurons excited by both IA and IB. The black squares represent neurons with multiple response patterns to IA and IB. The black triangles represent neurons responding to IA but having no response to IB. *E*: Schematic drawing of the T3 spinal segment (Molander et al. 1989). I-X indicates laminae; Liss, Lissauer's tract; LSN, lateral spinal nucleus; Pyr, pyramidal tract; IM, intermediolateral nucleus. IL, intermediolateral nucleus; CC, column of Clarke.

Fig 2.

Convergent patterns of pulmonary and cardiac inputs onto spinal neurons. *A*: a neuron with short-lasting excitatory responses (SL-E) to inhaled ammonia (IA) and intrapericardial bradykinin (IB). *B*: a neuron with long-lasting excitatory (LL-E) response to IA and IB. *C*: a neuron with inhibitory (I) responses to IA and IB.

Fig 3.
The effects of bilateral cervical vagotomy on spinal neuronal responses to IA and IB. *A–B*: Vagotomy did not affect excitatory responses of a spinal neuron to IA but reduced responses to IB in a spinal neuron. *C–D*: Vagotomy reduced excitatory responses to IA but enhanced response to IB in another spinal neuron.

Fig 4. Effects of intrapericardial lidocaine (2%, 0.2 ml, 10 min) on spinal neuronal responses to IA and IB. A, B, and C are spinal neurons responding to both of IA and IB and affected by intrapericardial lidocaine in different degrees.

Table 1

Comparison of excitatory responses of cardiopulmonary convergent neurons to both IA and IB (n=47).

Stimuli	Spontaneous activity (imp/s)	Latency (s)	Responses (imp/s)	Duration (s)
IA	8.1 ± 1.1	8.3 ± 0.5	20.4 ± 1.8	48.9 ± 5.0
IB	7.2 ± 1.1	5.0 ± 0.4 [*]	24.1 ± 2.5	129.5 ± 13.2 [*]

^{*} P<0.01 compared to corresponding responses to IA.

Table 2

Characterization of responses of cardiopulmonary convergent neurons to both IA or IB after cervical vagotomy.

Stimuli	Neuron #							
	1	2	3	4	5	6	7	8
IA	RR	NC	RR	ER	NC	NC	NC	NC
IB	RR	ER	ER	ER	ER	NC	RR	RR

RR, Reduction in responses. ER, Enhance in responses. NC, no change in response.

Reduced and enhanced responses was identified as a changes >20% of neuronal responses to IA or IB compared to those before vagotomy.

Table 3

Responses of cardiopulmonary convergent neurons to IA and IB before and after cervical vagotomy (n=8).

Stimuli	vagotomy	Spontaneous activity (imp/s)	Latency (s)	Responses (imp/s)	Duration (s)
IA	Before	7.6 ± 2.1	9.1 ± 1.5	17.8 ± 3.3	66.6 ± 16.2
	After	8.9 ± 3.5	10.7 ± 1.0	18.1 ± 3.0	48.7 ± 10.9
IB	Before	7.9 ± 2.5	6.2 ± 0.7	15.5 ± 2.5	136.8 ± 47.1
	After	7.6 ± 3.1	6.5 ± 1.0	17.3 ± 4.4	102.8 ± 12.5