Cardiac and Pulmonary Vagal Neurons Receive Excitatory Chemoreceptor Input

Chen-Tung Yen[1], Ji-Chuu Hwang[2] and Jong-Shin Wu[1]

[1]*Department of Zoology*
National Taiwan University
and
[2]*Department of Biology*
National Taiwan Normal University
Taipei 106, Taiwan, ROC

Abstract

The effects of hypercapnia and hypocapnia on the activities of the cardiac and pulmonary vagal single fibers were examined in the decerebrated, unanesthetized, paralyzed, and vagotomized cats. The animals breathed 100% O_2. Fractional end tidal CO_2 concentration was raised to 9% by adding CO_2 into the O_2 inlet. Average discharge rate of efferent cardiac vagal units (n=10) increased from 1.0±0.3 to 2.2±0.3 Hz. Hypocapnia apnea was produced by hyperventilation. Activities of cardiac vagal units tested (n = 4) showed dramatic decrease (0.1±0.0 Hz). Mean arterial blood pressure did not change significantly under these conditions. In contrast, only instantaneous firing rate during inspiration was significantly increased for efferent pulmonary vagal units (n = 11) during hypercapnia. The activities of the 3 pulmonary vagal units tested with hypocapnia decreased significantly. We concluded that cardiac and pulmonary vagal neurons were excited by chemoreceptor input.

Key Words: bronchoconstrictor, hypercapnia, hypocapnia, parasympathetic preganglionic neuron, single unit recording

Introduction

The heart and the lung are two of the most important organs of the body. Both organs are under the control of autonomic nervous system, of which, parasympathetic is one of its two major arms. Therefore, a clearer understanding of the parasympathetic control of the heart and lung should be of vital importance. Parasympathetic preganglionic neurons to the heart, i.e., cardiac vagal neurons, are known to receive excitatory baroreceptor input (14, 16). These neurons discharge during expiratory phase (15). Thus it seems likely that cardiac vagal activity should be modulated by chemoreceptor activity. The first purpose of the present study was to induce hypercapnia or hypocapnia conditions, and to test the effects of chemoreceptor modulation on cardiac vagal neurons.

Cardiac vagal nerve, or cardiopulmonary nerve, of the cat contains a mixed population of cardiac and pulmonary fibers (15). Very few studies have examined the functional properties of the pulmonary vagal neuron (15, 21). The second objective of the present paper was to record single pulmonary vagal fibers and to compare their functional properties with the cardiac vagal fibers. Chemoreceptor modulation is the most important control of pulmonary function. A direct recording of chemoreceptor modulation of efferent pulmonary vagal fiber was done in the present study by varying CO_2 concentration and observing effects on teased single pulmonary fibers.

Materials and Methods

Nine cats of either sex were used. These cats weighed 1.5 to 4.2 Kg. They were first anesthetized with halothane. Cannulations were performed on the femoral artery and femoral vein. After tracheal intubation, cats were decerebrated by bilateral ligature of the external carotid artery, followed by a

Corresponding author: Dr. Chen-Tung Yen, Department of Zoology, National Taiwan University, 1, section 4, Roosevelt St., Taipei 106, Taiwan, ROC. Tel: 886-2-2363-0231 ext. 3322, Fax: 886-2-2363-6837, E-mail: ctyen@ccms.ntu.edu.tw
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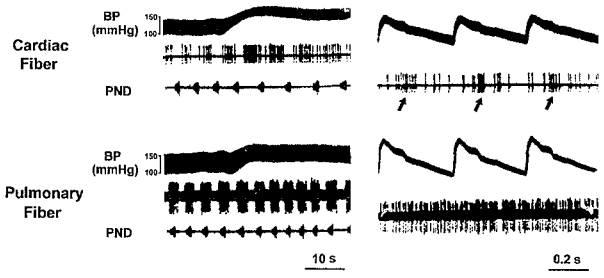

Fig. 1. Representative examples of the cardiac related activities in a cardiac vagal fiber (top panel). The firing rate of this unit increased during the blood pressure (BP) increase caused by i.v. phenylephrine (20 μg). When many synchronized BP pulses were superimposed, a clear cardiac rhythm (arrows) can be seen on the top right panel. Also showed on the lower panel is the negative response of a pulmonary vagal unit to the same manipulations. PND: phrenic nerve discharge.

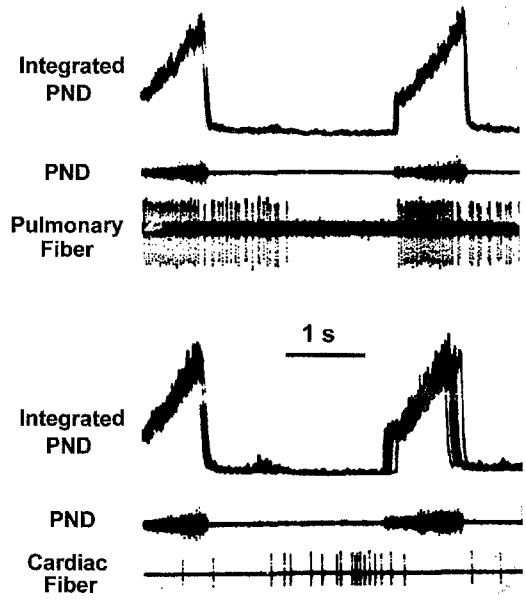

Fig. 2. Inspiratory (top panel) and expiratory (lower panel) -related activities of a pulmonary and a cardiac vagal units respectively. Superimposed oscillograms triggered by integrated phrenic nerve discharge (PND). Note the second burst of activities of the pulmonary vagal unit during the early expiratory phase.

midcollicular transection and removal of all rostral cortical and subcortical tissues (6). Afterwards, halothane was discontinued. There was a minimum of 6 h elapsed before actual data collection.

The cat was mounted on a stereotaxic apparatus. It was paralyzed with intravenous gallamine triethiodide and ventilated with 100% O_2. The fractional end tidal CO_2 concentration ($FECO_2$) was maintained between 3.8 to 4.2 %. The rectal temperature of the cat was maintained between 36 to 37 °C with a thermal blanket and a heating lamp.

The right phrenic nerve was isolated by a lateral approach at the lower neck level. The chest was opened by removing the lateral part of the 1st to the 5th rib. Bilateral vagotomy was performed by transecting the left cervical vagus and the right thoracic vagus. Isolation and identification of the right cardiac sympathetic and the right cardiac vagal nerves have been described previously (22). Briefly, all outputs from the right stellate ganglion were cut. Either the ventral ansa or the inferior cardiac nerve (19, if present) were prepared for whole nerve recording of the cardiac sympathetic activity. Usually two cardiopulmonary nerves (caudovagal nerves) were found near the right atrium after the azygous vein was tied and transected (15, 19). These nerves were isolated from the surrounding tissue and cut distally. A piece of the thoracic vagus about 3 - 4 cm long was isolated along with the cardiac vagal nerves and placed in an oil pool.

A tease fiber method was use to obtain single fiber recording of the central cut end of the cardiopulmonary nerves (6). Activities from a single fiber were judged by the similar height and waveform of the action potentials. Criteria established by McAllen and Spyer (15) were used to distinguish cardiac and pulmonary vagal fibers. Namely, the cardiac fiber (1) had a cardiac rhythm (Fig. 1, top right); (2) increased its firing rate during an increase in blood pressure (Fig. 1, top left); and (3) discharged with an expiratory rhythm (Fig. 2B). Whereas the pulmonary fiber discharged with an inspiratory rhythm (Fig. 2A) without discernible cardiac rhythm (Fig. 1, lower panel). Conventional electrophy-siological techniques were used to record and monitor whole nerve activities of phrenic and cardiac sympathetic nerves (band pass filter set at 300 - 3K and 1 - 3K Hz respectively). Blood pressure and expiratory CO_2 concentration were monitored continuously and stored along with the neural records with a tape recorder (model 886, Neurodata).

Hypercapnia was induced by mixing CO_2 gas into the respired O_2. Periods of 5 to 10 minutes were allowed for the adjustment of $FECO_2$ to 9%. For each unit recorded, 6 min data each were collected during the pretest control period, during the 9% hypercapnia, and the control period afterwards when the $FECO_2$ level returned to 4%. In 4 cats, an additional hypocapnia test was conducted. The hypocapnia condition was induced by hyperventilation till phrenic activity was totally suppressed. This test was given subsequent to the hypercapnia test. About 2 min period of stable hypocapnia data was collected. Another 6-min post-stimulus control data was collected after the $FECO_2$ level had returned to 4%.

Data analysis was done off-line. Single unit activities were preprocessed with a window discriminator. Whole nerve activities and TTL pulses from the window discriminator were integrated with

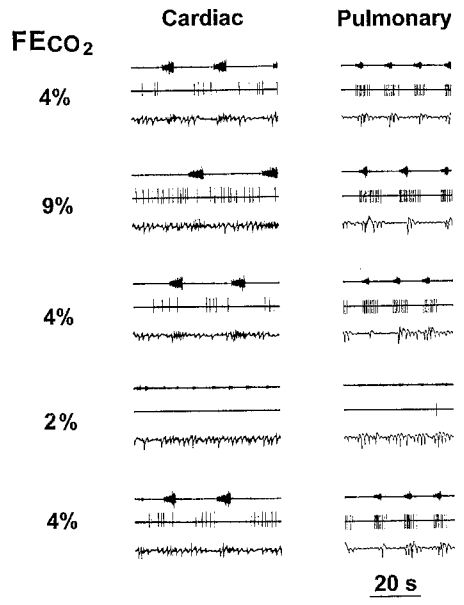

Fig. 3. A cardiac vagal unit and a pulmonary unit (from two animals) are shown in left and right panels, respectively. Within each display are phrenic activity (top trace), single unit activity (middle trace) and cardiac sympathetic activity (lower trace) under normocapnia ($FECO_2$ = 4 %), hypercapnia (9 %) and hypocapnia (2 %). Experiments were performed from topmost to lowermost sequence.

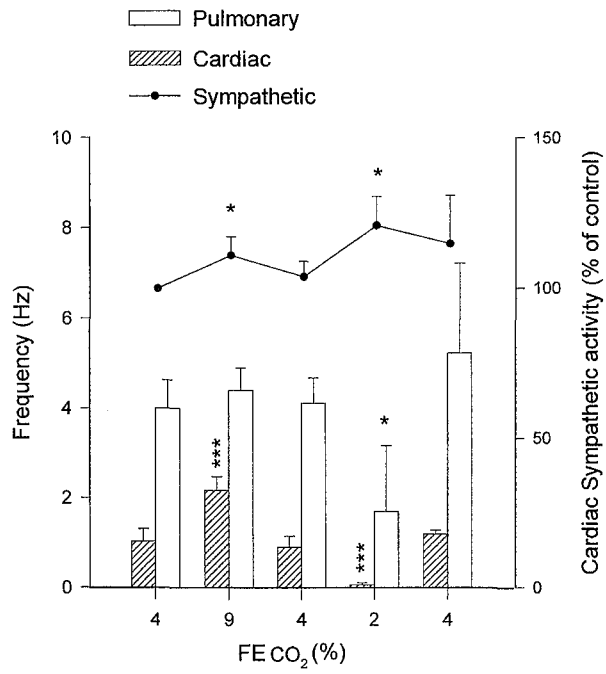

Fig. 4. Averaged changes of pulmonary vagal unit (open bars), cardiac vagal unit (hatched bars) and cardiac sympathetic nerve (solid dots) activities to changes in $FECO_2$, from normocapnia (first 4 %), to hypercapnia (9 %), back to normocapnia (second 4 %), to hypocapnia (2 %), and back again to normocapnia (third 4 %). *: P< 0.05; ***: P< 0.001; compared with pre-stimulation control.

a RC integrator (time constant 50 ms). Six or 2 min (hypocapnia) integrated neural signals, blood pressure and CO_2 concentration data segments were digitized at 512 samples/s using an A/D converter (MP100, BIOPAC System Inc., Goleta, CA). Spike count and average nerve activities were calculated with commercial software (Acqknowledge, BIOPAC System Inc.). Paired t-test was used to compare pre- and post-stimulation conditions. $P<0.05$ was considered significant. Data were expressed as mean ± standard error unless specified otherwise.

Results

Thirty-one single unit were recorded in the cardiopulmonary nerve. Of these, 10 were cardiac, 16 pulmonary, and the other 5 could not be fitted into these two categories. All the pulmonary vagal fibers had in addition to the inspiratory rhythm, extra discharges during the early expiratory phase (Fig. 2A). Hypocapnia was tested on 4 cardiac and 3 pulmonary fibers. Cardiac vagal fibers were strongly activated during hypercapnia, and suppressed during hypocapnia. For example, the cardiac fiber shown in Figure 3 discharged 15 spikes during the illustrated control period of 60 s (0.25 Hz). Its firing rate doubled (0.5 Hz) during hypercapnia. This excitation was reversible. The firing rate of this neuron returned to 0.25 Hz after $FECO_2$ had returned to 4%. Conversely, hypocapnia produced a profound decrease in the firing rate of this neuron. In the whole 2 min of recording period, not a single spike was appeared. This inhibition was also reversible (Fig. 3 lowest panel). Results from all units tested were averaged and shown in a bar graph (Fig. 4). Spontaneous firing rate of the 10 cardiac vagal fibers averaged 1.02±0.28 Hz. It increased significantly to 2.16±0.31 Hz during hypercapnia and decreased significantly to 0.05±0.05 Hz (in 4 of the 10 units tested) during hypocapnia. The average blood pressure levels for the 5 conditions were 117±13, 111±16, 108±13, 101±16 and 110±16 mmHg respectively. There was no significant difference among these values.

In comparison, pulmonary vagal fibers showed similar trend of response to hypercapnia and hypocapnia stimuli. The responses, however, were smaller. An example is shown on in Figure 3 (right panel). This unit discharged 43 spikes during the 40-s control period. When $FECO_2$ increased to 9%, instantaneous firing rate in the inspiratory phase increased greatly. The total number of spikes, however, increased only slightly to 53. This unit showed a marked decrease in its firing rate during hypocapnia (firing rate dropped to 0.65 Hz in the 2 min tested period). Other pulmonary units showed milder reaction to the same hypocapnia stimuli (Fig.

4, open bars). The averaged firing rate of the 11 units tested in response to hypercapnic condition was not significantly different from those to normocapnia. Maximal instantaneous firing rate during inspiration was measured and averaged for 20 inspiratory cycles for each condition. The average maximal instantaneous firing rate was 13.0±2.1 Hz during normocapnia. It increased significantly to 15.2±1.8 Hz during hypercapnia. Three pulmonary units were tested with hypocapnia. Their spontaneous firing rate showed significant decrease compared with pre-stimulation control (1.67±1.49 vs. 5.48±1.72 Hz).

Cardiac sympathetic activities were recorded simultaneously in every animal. Examples from two cats are illustrated in Figure 3, one with cardiac vagal fiber, the other with pulmonary fiber activities. With wide-band (1 Hz - 3K Hz) recording condition, cardiac sympathetic activity had respiratory and cardiac rhythms. These fluctuations persisted during either enhanced or depressed ventilatory drives in hypercapnia or hypocapnia respectively. Averaged sympathetic activities were higher in both hypercapnia and hypocapnia. During hypercapnia, inspiratory fluctuations appeared larger. During hypocapnia, continuous fluctuations synchronized to the blood pressure waves were seen without quiescent periods usually observed during both normocapnia and hypercapnia. These increases were statistically significant when data from all experiments were compared (Fig. 4).

Discussion

The present paper showed a strong correlation between cardiac vagal activity and central respiratory drive. Cardiac vagal activity increased two folds during hypercapnia in normoxia and almost dropped to zero during periods when phrenic activities were totally suppressed by hypocapnia. Similar but milder responses were found in pulmonary vagal neurons. In contrast, cardiac sympathetics were excited during both hypercapnia and hypocapnia.

Before discussing the significance of our findings, several technical issues should be addressed. These issues are the effects of anesthesia and techniques used in activating chemoreceptors. Tonic vagal activity to the heart is very low in anesthetized cat (15, 22). Most of the single fiber studies were done in the dog (4, 7, 9, 10, 11, 12, 14, 20). It is a noteworthy technical discovery that a much higher cardiac vagal tone existed in the decerebrated and unanesthetized cat. Iscoe and Fisher (8) examined specifically what effects anesthetics would produced on the pulmonary mechanics in decerebrated and unanesthetized cat. They found a significant bronchomotor tone in unanesthetized cat. This tone is muscarinic in origin (most likely vagal) and increase with hypercapnia. Our data here on the pulmonary vagal activities underlie well their pulmonary data. Therefore, data from the present study suggest that the decerebrated, unanesthetized cat is a very useful animal model not only for the studying of the bronchomotor tone, pulmonary vagal activity, but also for cardiac vagal activity.

Hypercapnic condition was obtained by mixing CO_2 gas into the respired O_2. Therefore, our condition was a hypercapnia in normoxymia. Although we did not measure blood CO_2 concentration, similar preparation by Iscoe and Fisher (8) obtained an arterial CO_2 level of 27 mmHg for their normocapnia condition and 41 mmHg when they mixed 5% CO_2 in the respired air. Dickstein et al. (3) varied $FECO_2$ level systematically from 4 % to 12%. They found a proportional change in P_aCO_2, ranged from 30-60 mmHg at 4% and 50-80 mmHg at 12%. Calculation based on their data yields an average of 35 mmHg at 4% and 62 mmHg at 9%. It is not unreasonable to assume a similar proportional change of arterial blood CO_2 level in our hypercapnic condition. We believe that hypercapnia at this level should be very effective in activating the central and the peripheral chemore-ceptors although aortic nerve receptors were removed. Our preliminary data showed strong baroreceptor input to medullary unit and vagal efferent fibers (Fig. 1). This is a good indication that both central and peripheral baro- and chemo-receptors were func-tioning.

The cardiac vagal neurons are activated by baroreceptors and they discharge in synchrony to the blood pressure within normal pressure range (16, Fig. 1 in the present paper). Activities of these neurons are inhibited during inspiration (2, 20). It is very interesting to note that this inhibition was not absolute during periods of enhanced inspiration (Fig. 3, left panel). On the other hand, cardiac vagal activities vanished during apnea induced by hyperventilation. These data suggested that inspiratory inhibition might not be as important in the determination of the cardiac vagal activity as chemoreceptor inputs, either central or peripheral in origin. In fact, it has been shown in various laboratories that stimulation of chemoreceptor in the dog strongly activates cardiac vagal neurons (2, 11, 12). Our data strongly support that the same excitatory connection may also exist in the cat. Another explanation of the strong cardiac vagal changes seen with alternation in $FECO_2$ might be a strong central coupling of the cardiac vagal neurons with the central respiratory rhythm generator. Because chemoreceptor strongly excites the central respiratory network, results in the present study cannot differentiate these two possibilities.

There have been even fewer direct studies of the pulmonary vagal neurons (15, 21). McAllen and

Spyer (15) recorded pulmonary vagal units in anesthetized cat. They found that these neurons discharged spontaneously during inspiratory phase (I neuron) and they had no cardiac related activity. In decerebrated, unanesthetized cat we found the firing pattern of these neurons were of the I-E type, i.e., in addition to the inspiratory activity a second burst of discharges in the early expiratory phase (6). It is noteworthy that the I-E firing pattern is very similar to those fibers found in the recurrent laryngeal nerve (Hwang et al., unpublished data), which is also a thoracic vagal branch. Whether a prominent expiratory activity is a distinguishing characteristic of the thoracic respiratory-related nerve and what the functional significance this will imply is an interesting topic for future studies.

Most of knowledge of the neural control of the lung derived from indirect observation of the bronchial resistance (3, 5, 8, 13, 17). In unanesthetized cats, there is a good bronchial tone (8). Muscarinic blocker decreases this by 50% (8). Ventilation of cats and dogs with carbon dioxide-rich gas mixtures constricts bronchial tree. This effect is abolished by vagotomy (1, 17) or atropine (3). Data in the present study is a direct confirmation of the neural involvement in the bronchial tone and the reflexive bronchial response to hypercapnia. In contrast, it has been shown that in voluntary human subjects, hyperventilation-produced hypocapnia causes also an increase in flow resistance, i.e., a bronchial constriction. Whether this effect is neurally-mediated is controversial (1). Data in the present study on decreased pulmonary activity during hyperventilation will be a piece of evidence added to the side of a local mechanism.

In summary, single unit data in the present paper support the hypothesis that cardiac and pulmonary vagal neurons of the cat receive excitatory chemoreceptor input. Whether this input originates from the central or the peripheral chemoreceptors requires further investigation.

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