SPECTRUM OF MYELINATED PULMONARY AFFERENTS (II)

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Abstract

Recently, it has been recognized that a single airway sensory unit may contain multiple receptive fields, and that each field houses at least one encoder. Since some units respond to both lung inflation and deflation, we hypothesized that these units contain heterogeneous encoders for sensing inflation and deflation, respectively. Single unit activities were recorded from the cervical vagus nerve in anesthetized, open chest, and mechanically ventilated rabbits. Fifty two airway sensory units with multiple receptive fields and responding to both lung inflation and deflation were identified. Among them, 13 units had separate receptive fields for inflation and deflation, where one of the fields could be blocked by local injection of 2% lidocaine (10 µl). In 8 of the 13 units, the deflation response was blocked without affecting the unit’s response to inflation, whereas in the remaining 5 units, the inflation response was blocked without affecting the deflation response. Our results support the hypothesis that a single mechanosensory unit may contain heterogeneous encoders that can respond to either inflation or deflation.

Keywords: vagus nerve; sensory receptors; deflation receptor; lung afferents; airway receptors
Introduction

Two types of mechanoreceptors, which can respond to lung inflation, exist in the airways: rapidly adapting receptors (RARs) and slowly adapting receptors (SARs). They transmit mechanical information through myelinated afferents in the vagus nerve (5; 13; 15; 18). Though the behaviors of RARs and SARs are unique, they may demonstrate similarities (20). Thus, these myelinated pulmonary afferents were proposed being connected to a heterogeneous group of receptors, which behave as a spectrum with typical SARs and RARs represent two extremes of the spectrum (20). Some afferents only respond to lung inflation, yet others respond to both inflation and deflation (2; 19).

Conventionally, the responses to inflation and deflation are explained by a single receptor hypothesis, in which stimuli such as lung inflation or deflation are encoded by a single receptor (RAR or SAR) (1; 5; 9; 11; 13; 15; 16; 19; 20). Alternatively, this phenomenon can be explained by a multiple receptor hypothesis (21), in which different stimuli are encoded by different receptors. Recently, a single pulmonary mechano-sensory unit was found to possess multiple receptors (23). A sensory receptor is an encoder, which is the smallest device that can independently generate action potentials. A receptive field is a sensory region that houses receptors. A receptive field contains at least one receptor but often has multiple receptors. The discovery of multiple receptive fields in a single unit (23), along with multiple sensory structures connected to a single axon (22), leads to the hypothesis that an airway mechano-sensory unit is a functional unit that contains multiple receptors with different characteristics. Each is capable of sensing one type of mechanical stimuli, such as lung inflation or deflation. Furthermore, each may detect a static (slowly adapting) versus a dynamic (rapidly adapting) component for inflation or deflation. Thus,
four different types of encoders exist: rapidly adapting inflation, slowly adapting inflation, rapidly adapting deflation, and slowly adapting deflation encoders. In the current studies, we tested this hypothesis by recording activity in a single unit that responded to both lung inflation and deflation. If we identified more than one receptive field, we injected local anesthetic into one of the fields to see if inflation or deflation response could be eliminated. Indeed, the present results show that inflation and deflation responses could be blocked independently, supporting that there are heterogeneous encoders within a single sensory unit.

Methods

General. Experiments were carried out in male New Zealand white rabbits intravenously anesthetized with 20% urethane (1g/kg). Additional doses (one seventh of the initial dose) were given whenever necessary. Study procedures were in accordance with ethics codes set by the NIH and approved by the IACUC of the University of Louisville. A midline incision was made to expose the trachea and vagus nerve. The trachea was cannulated low in the neck close to the carina and the lungs were mechanically ventilated with a Harvard ventilator (Model 683, South Natick, MA). Positive-end-expiratory pressure (PEEP) was maintained by placing the expiratory outlet under 3-4 cm H₂O. Airway pressure was monitored at the tracheal tube inlet by a Statham pressure transducer (P23). The chest was opened widely in the midline to allow locating the receptive field during single unit recording (see below). Unit responses to cyclic changes in airway pressure, PEEP removal, and lung inflation were examined before and after microinjection of local anesthetic (2%
lidocaine). Lidocaine was injected into the receptive field with a needle (30 GD) in 10 µl. Airway pressure and afferent activities were recorded by a thermorecorder (Astro-Med Dash IV).

**Recording of afferent activity.** Single-unit activities from vagal afferents were recorded according to conventional methods (20). The vagus nerve (either right or left) was separated from the carotid sheath, placed on a dissecting platform, and covered with mineral oil. A small afferent bundle was cut from the vagus nerve, and was dissected into thin filaments with two pairs of fine forceps. The filaments were further divided and placed on recording electrodes to measure action potentials. The electrodes were connected to a High Impedance Probe (Grass Model HIP 511), from which the output was fed into an amplifier (Grass P 511). After suitable amplification, action potentials from a single-unit of the vagal sensory receptors were displayed on an oscilloscope and monitored by a loudspeaker. In addition, a voltage analogue of impulse frequency was produced by a rate meter (Frederick Haer, Brunswick, ME) at a band width of 0.1 sec. Adaptation rate was determined by an adaptation index.

**Sensory unit identification.** The mechanosensory units that responded to both lung inflation and deflation were identified (20). Then, the receptive field was located by identifying the most sensitive point on the lung surface with a glass rod having a 0.5 mm round tip. At the center of this point, touching elicited a high discharge frequency, which can be easily identified by listening to the monitor and hearing a high pitch burst of action potentials. This response subsided, as the distance away from the center increased.
The sensory units containing more than one receptive field were further examined. That recorded activities were coming from a single unit was verified by inspection of the contour of action potentials with the oscilloscope under a high time resolution and by their discharge pitch from the loudspeaker. All receptive fields identified were in the lung parenchyma. It needs to be point out that lung collapse is a heterogeneous condition. The response of a receptor to collapse depends on its local conditions as well as inherent properties. For example, a deflation activated receptor (DAR) may not respond to lung deflation if it is located in a lung region where air is trapped. In addition, RARs usually have a very low discharge frequency during normal tidal ventilation. An active search for RARs requires large inflations or deflations with negative pressure for each time a new strand of vagal afferents is put on the electrodes for recording. In the current studies we did not perform such maneuvers, therefore, our studies would have under-counted the percentage of RARs in the myelinated afferent population.

**Procedures.** When a sensory unit had been identified, the lung was deflated by removal of PEEP for 4-5 ventilatory cycles. If the units responded to PEEP removal, they were entered for a series of procedures. First, the lung was inflated to 30 cm H_{2}O constant pressure, then the lung was deflated to –7 cm H_{2}O. The unit responses to these pressures were used to calculate an adaptation index. Adaptation index is the difference between discharge frequencies of the peak and average of the second sec, which is then divided by the peak frequency and expressed as percent. Peak discharge usually occurs within the first 0.3 seconds after pressure reached peak. Any units with a delayed discharge peak were excluded from the study. An adaptation index above 70% is defined as rapidly
adapting. Then, the receptive fields would be identified again. Local anesthetic (2% lidocaine) was injected directly into one of the fields to determine whether the inflation or deflation response can be blocked. The deflation response was assessed by counting the number of action potentials in a complete deflation phase during the second ventilatory cycle after PEEP removal. We used PEEP removal for the quantitative analysis because this maneuver is simple, reproducible and precise. The inflation response was assessed by counting the number of action potentials for one second after the first second following initiation of lung inflation.

Results

In 42 rabbits, we encountered 52 mechanosensory units (1-2 units per rabbit) that responded to both lung inflation and deflation, and had separate receptive fields. Three of the 52 units were rapidly adapting, and the remaining units were slowly adapting ones. Therefore, our study is mainly about slowly adapting units. Activities of these units increased during the lung inflation phase and decreased during the deflation phase. The activities increased again in response to lung deflation either by PEEP removal or negative pressure deflation. Upon constant pressure deflation, ten of the units adapted rapidly. The adaptation rate was higher than that to lung inflation (Fig. 1). If rapidly adapting units (either to lung inflation or deflation) were removed, the adaptation rate to lung deflation was still higher than to lung inflation. There was no correlation between the two adaptation indexes. For any given unit, the adaptation index for lung inflation can be lower, higher, or the same as for lung deflation (Fig. 1).
Among the 52 units, activities in 10 units were blocked completely by local injection of lidocaine into one of the receptive fields; activities in 29 units decreased both in response to lung inflation and deflation. In these 29 units, however the suppressive effects varied from unit to unit. The suppressive effects could be more on inflation, or deflation, or equally. Figure 2 illustrates simultaneous suppressive effects. However, in the remaining 13 units, only one of the responses was blocked or significantly decreased by injection of lidocaine into one of the receptive fields. In 8 of the 13 units, the deflation response was significantly blocked without substantial effects on the inflation response (Fig. 3), whereas in the remaining 5 units, the inflation response was greatly attenuated with little effect on the deflation response (Fig. 4). The grouped data for these 13 sensory units are illustrated in Fig. 5.

Figure 6 illustrates a unit that clearly had both rapidly adapting and slowly adapting components in response to lung inflation, i.e., RAR and SAR shared an axon. Local injection of lidocaine blocked the RAR without affecting the SAR activity in the sensory unit. Figure 7 shows a unit that contained multiple heterogeneous encoders, including inflation and deflation activated encoders. Both inflation and deflation responses had rapidly adapting and slowly adapting components. This unit has an active DAR, which is blocked by local anesthetic without affecting the inflation response. Such a unit is not popular in the rabbit but is popular in the rat (Fig. 8 and (3; 17)). Figure 8 illustrates that an active DAR was not affected by blocking the inflation encoder in a sensory unit from a rat.
Discussion

Our current studies demonstrated that responses of a mechano-sensory unit to both lung inflation and deflation could be blocked independently, indicating that they are coming from different receptors.

Sensory afferents that respond to lung deflation were first identified in a multiple fiber preparation by Keller and Loeser in the rabbit (8), and then in a single fiber by Adrian in both the rabbit and the cat (1). These sensory units also responded to lung inflation, but often at a slowly adapting rate. Twenty (19) to 30 percent of the slowly adapting units has been reported to respond to forced lung deflation (9). In our previous study 11% of slowly adapting units responded to lung deflation with removal of PEEP (20). This type of units has been studied extensively in the rabbit (1; 11; 14), and other animals such as cats (1; 9; 19), dogs (2), guinea pigs, (10) rats (3; 6; 12; 17) and mice (25). Airway sensory units can be slowly or rapidly adapting to either lung inflation or deflation. Adaptation indexes to lung inflation and deflation can be similar or different but they behave independently. Currently, investigators categorize sensory units into rapidly or slowly adapting ones according to their response to lung inflation. The differences in adaptation indexes to lung inflation and deflation justify further separation into groups according to their stimulus.

Independent blockade of a sensory response to inflation or deflation suggests that the activities come from different encoders. We do not know yet if one encoder can respond to both inflation and deflation. However, we provide compelling evidence that
the inflation response is separate from the deflation response, and generated from two separate encoders. For example, as unit response to deflation was blocked, the response to inflation was unchanged (Figs. 3 and 5), and vice versa (Figs. 4 and 5). Such independent blockade was also demonstrated in the rat (Fig. 8). This supports our theory that a mechano-sensory unit consists of many encoders and the mixed behavior is due to the heterogeneous composition of encoders. The sensory unit in figure 7 may possess all the 4 receptor types. This is demonstrated by that when the lung was inflated to 30 cmH$_2$O or deflated to -7 cmH$_2$O, the unit discharged immensely and discharging frequency adapted rapidly to a steady state level with no further significant adaptation. Since each encoder has its own characteristics in activation threshold, operation range, operating sensitivity, and deactivation threshold, within a type individual encoder behaves similarly, but not identical. When a sensory unit consists of homogenous encoders, it behaves as a typical SAR, RAR, or rapidly adapting or slowly adapting DAR. However, a unit may contain heterogeneous encoders, exhibiting mixed behavior. Thus, it may respond to both inflation and deflation, or behave as RAR-like SARs (Fig. 6) (20). In addition to Fig. 6, Figs 2, 4, 7 also demonstrate rapidly adapting and slowly adapting components during lung inflation to 30 cmH$_2$O, indicating co-existence of RAR and SAR in the sensory unit. Co-existence of RAR and SAR in a sensory unit has been found in mammalian muscle spindle (7). Similarly, many units had the rapidly and slowly adapting components during constant pressure deflation (Figs. 3 and 7). Interestingly, the slowly adapting deflation response could be selectively blocked without affecting rapidly adapting deflation response (Fig. 3).
An airway sensory afferent may connect to many receptive fields, which was found in about one third of slowly adapting units (23). Multiple receptive fields were also found in sensory units in the other visceral organs, such as esophagus, stomach and colon (4; 24). Morphologically, receptor structures may be as close as a few hundred μm (21). The techniques used in the current studies can not identify two receptive fields less than 1 mm apart (23). Thus, a receptive field may contain multiple encoders. Multiple encoders within a receptive field may explain simultaneous suppression of inflation and deflation responses when one of the receptive fields was blocked. Alternatively, local injection is not precise and the distribution of local anesthetic is complicated. Liquid may be blocked by alveolar wall from infiltrating alveoli nearby, yet run through airways to more distant sites. Furthermore, the anesthetic may spread to adjacent areas. This may explain why blocking one receptive field may abolish the sensory activity.

In conclusion, our data support the contention that an airway mechano-sensory unit consists of many encoders. Each encoder has a special function to detect a unique variable, such as inflation, deflation, static stimulation, or dynamic stimulation. When a sensory unit consists of homogenous encoders, it behaves as a typical SAR, RAR or DAR, whereas when a sensory unit contains heterogeneous encoders, it exhibits mixed behavior. Significant amount information is integrated at the sensory unit level. Thus, airway sensory units function not only as transducers but also as processors.

Perspectives
Airway mechanosensors provide the respiratory centers with crucial information about lung mechanics for controlling breathing. Therefore, a thorough understanding of mechanosensory behavior is necessary. Learning the pattern of the sensory discharges has attracted the attention of many investigators for more than a half-century, however, our understanding is still incomplete. The current results support a theory that an airway mechanosensory unit contains multiple receptors, including rapidly adapting receptors, slowly adapting receptors, and deflation activated receptors. Each type of receptor is capable of sensing a particular variable of lung mechanics. Thus, the sensory unit is not only a transducer but also a processor that integrates a significant amount of information. This new insight will challenge our current view regarding how the central nervous system deciphers the incoming signals from the respiratory system.

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Reference List


Figure legends:

Fig. 1. Correlation between sensory units’ inflation and deflation adaptation index (AI, R=0.205, P=0.1446, n=52). Each symbol represents a sensory unit. Black square denotes slowly adapting units; open circle denotes rapidly adapting units. Clearly, these sensory units were more rapidly adapting in response to lung deflation (-7 cm H2O) than inflation (-30 cm H2O), demonstrated by more units above the equal value line, with an average adaptation index (51±19% for lung deflation and 34±16% for lung inflation; P<0.001, n=52). Such a relationship remains after removing all the rapidly adapting units (circular ones), when averaged AI was 46±16% for lung deflation and 34±14% for lung inflation (P<0.001, n=39).

Fig. 2. Blocking one receptive field may simultaneously suppress inflation and deflation receptors. A typical slowly adapting unit recorded from the left cervical vagus nerve in an anesthetized, open-chest, and mechanically ventilated rabbit. This unit has two separate receptive fields. The traces are: IMP/s, sensory activity per second counted at each 0.1 second; IMP, impulses or action potentials; Paw, airway pressure measured in cm H2O. A-C, intact, controls; D-F, block, after blocking one field. PEEP removal (A, D); Inf 20 cmH2O and Inf 30 cmH2O, lung inflation to a constant pressure of 20 and 30 cmH2O,
respectively. Please note that though both inflation and deflation responses were attenuated, none was eliminated.

Fig. 3. Blocking one receptive field blocked deflation response but not inflation response. A-D, intact (pre-controls); E-H, block (after blocking one of the receptive fields); I-L, recovery (post-controls). They were tested under PEEP removal (A, E, I), lung deflation at the pressure of -7 cm H₂O (B, F, J), lung inflation at pressure of 20 (C, G, K) and 30 (D, H, L) cm H₂O, respectively. Under the control conditions, after PEEP removal the unit discharged during the expiratory phase (A), and was vigorously stimulated by deflation (B). Please note that the sensory unit responded to negative pressure deflation with 2 phases, an initial rapidly adapting phase followed by a slowly adapting phase. After blocking one of the receptive fields, the unit response to PEEP removal (E) and slowly adapting component in response to the negative pressure deflation (F) disappeared, leaving rapidly adapting component intact. However, the unit response to lung inflation did not change (G, H). For Figure legend abbreviations, please see Figure 2.

Fig. 4. Blocking one receptive field blocked inflation response but not deflation response. Sensory unit activities were recorded before (A, B, C, D) and after (E, F, G, H) blockade of one receptive field with 2% lidocaine (10µl). The expiratory activity did not change, but the inspiratory activity was significantly decreased after the blockade. For Figure legend abbreviations, please see Figure 2.
Fig. 5. Effects of lidocaine to block inflation or deflation responses in sensory units. The left four columns are grouped data for the blockade of inflation responses. The right four columns represent the data for the blockade of deflation responses (i.e., response to PEEP removal). The solid columns are deflation responses. The blank columns are inflation responses. * denotes p<0.05; ** denotes p<0.01. Columns 1 and 3 are controls, whereas 2 and 4 are after blockade. Please note that when the inflation response was blocked, the deflation response did not change substantially (left columns). Similarly, when the deflation response was blocked, there were no significant effects on inflation responses (right columns).

Fig. 6. SAR and RAR sharing an axon. This sensory unit has two heterogeneous encoders: SAR (low threshold and low frequency) and RAR (high threshold and high frequency). In A, during lung inflation, the unit starts with SAR activation, producing a low discharge frequency; as the airway pressure increases, the RAR is activated (indicated by two arrows) and the unit discharges with very high frequency. During constant lung inflation, the unit fires with a higher frequency (from the RAR encoder) initially, but rapidly adapts to a steady state (from the SAR encoder). In B, blocking the RAR encoder with local injection of 2% lidocaine, the rapidly adapting component disappeared and left only the slowly adapting component, which exhibited continuous lower discharge frequency. For Figure legend abbreviations, please see Figure 2.

Fig. 7. A sensory unit with an active deflation activated encoder, i.e., it was active during lung deflation phase (indicated by arrows), which can be selectively blocked. A-E are
controls with an intact sensory unit, showing PEEP removal, negative pressure deflation, and constant pressure inflation of the lung to 10, 20, 30 cm H2O, respectively. F-J were recorded after lidocaine blockade of one of the receptive fields. The active deflation activated encoder was blocked. However, the unit was still activated during a strong deflation either by PEEP removal (F) or by negative pressure (G), although activity was significantly reduced. Clearly, the active deflation encoder has a low activating threshold and this sensory unit contains more than one deflation encoder. For Figure legend abbreviations, please see Figure 2.

Fig. 8. A sensory unit with an active deflation activated encoder from a rat. This unit is active during both inflation phase and deflation phase under resting mechanical ventilation. This unit has two receptive fields with one responding to inflation and the other to deflation. Please note that the inflation activity was blocked but the deflation activity did not change after lidocaine. For Figure legend abbreviations, please see Figure 2.
Unit activity (impulses)

- Inflation
- Deflation

Inflation block (n=5)

Deflation block (n=8)