Airway Mechanosensor Behavior during Application of Positive End-Expiratory Pressure

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Key Words

Cellular mechanotransduction · Mechanosensors · Positive pressure respiration · Sensory receptors · Vagal afferents

Abstract

Background: Positive end-expiratory pressure (PEEP) is commonly used in clinical settings. It is expected to affect the input from slowly adapting pulmonary stretch receptors (SARs), leading to altered cardiopulmonary functions. However, we know little about how SARs behave during PEEP application. **Objectives:** Our study aimed to characterize the behavior of SARs during PEEP application. **Methods:** We recorded single-unit activities from 18 SARs in the cervical vagus nerve and examined their response to an increase of PEEP from 3 to 10 cm H₂O for 20 min in anesthetized, open-chest and mechanically ventilated rabbits. **Results:** The mean activity of the units increased immediately from 35.7 to 80.5 impulses per second at the fifth breath after increasing PEEP (n = 14, p < 0.001) and then gradually returned to 56.5 impulses per second at the end of 20 min of PEEP application (p < 0.001). In the meantime, peak airway pressure increased from 9.3 to 32.7 cm H₂O, and then gradually returned to 29.4 cm H₂O (n = 18; p < 0.05) after 20 min. The remaining four units ceased firing at 34.7 s (range 10–56 s)

after their initial increased activity upon 10 cm H₂O PEEP application. The unit activity resumed as the PEEP was returned to 3 cm H₂O. **Conclusions:** High PEEP stimulates SARs and SAR activity gradually returns towards the baseline via multiple mechanisms including receptor deactivation, neural habituation and mechanical adaptation. Understanding of the sensory inputs during PEEP application will assist in developing better strategies of mechanical ventilation.

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Background

Airway sensory receptors play an important role in the control of breathing and airway caliber [1–3]. The activation of airway mechanosensors (mechanoreceptors) – called slowly adapting pulmonary stretch receptors (SARs) – initiates the Hering-Breuer reflex (HBR) [4], through which signals are sent to the central nervous system to trigger expiration. Positive end-expiratory pressure (PEEP) is commonly applied during mechanical ventilation in critically ill patients and during noninvasive mechanical ventilation using either bilevel positive airway pressure (BPAP) or continuous positive airway pressure (CPAP) [5, 6]. In acute respiratory distress syn-

drome, a high PEEP is employed to recruit collapsed alveoli in dependent regions of the lung to improve V/Q mismatch [7]. PEEP is also used in spontaneous breathing trials during ventilator weaning and in obstructive sleep apnea. Tryfon et al. [8] applied PEEP to healthy subjects and patients with chronic obstructive pulmonary disease (COPD) or interstitial fibrosis, and examined the HBR. The HBR was augmented in interstitial fibrosis (decreased lung compliance), but was unaffected in COPD (chronic intrinsic PEEP), even at a PEEP level of 20 cm H₂O [8]. In 1975, Stanley et al. [9] demonstrated that phrenic nerve activity was inhibited by application of a 10-cm H₂O PEEP, which was independent of blood gases. The inhibition rebounded immediately as the PEEP was removed. Expiratory time also increased as the PEEP level increased; however, with continued PEEP, the expiratory time returned towards baseline [10]. Despite the frequent use of PEEP in clinical practice, little is known about the neurophysiological effects of high PEEP, particularly its interactions with SARs. The purpose of this study was to elucidate the effects of PEEP on SAR discharge, which may be critical in the control of breathing.

Materials and Methods

General

All procedures complied with the ethical standards established by the National Institutes of Health and were approved by the internal Animal Use and Care Committee of the University of Louisville, Ky., USA. Fifteen New Zealand White male rabbits (2.0–2.3 kg) were anesthetized with 20% urethane (1 g/kg, i.v., Sigma-Aldrich, St. Louis, Mo., USA). After midline incision to expose the trachea and vagus nerve, the trachea was cannulated low in the neck. The lungs were ventilated at 10 ml/kg tidal volume with a rate of 30 cycles/min by a Harvard ventilator (Model 683, South Natick, Mass., USA), maintaining PEEP by placing the expiratory outlet under 3 cm H₂O. Airway pressure was monitored by a Stat-ham pressure transducer (P23) attached to the tracheal tube. The chest was then opened wide along the midline so that the receptive field could be located by gently exploring the pleural lung surface with a cotton tip. Receptor responses to cyclic changes in airway pressure and PEEP were examined. Airway pressure and afferent activities were recorded by an Astro-Med thermorecorder (Dash IV), recording SAR single-unit activities and responses to increasing PEEP from 3 to 10 cm H₂O over a course of 20 min.

Recording Afferent Activity

SAR receptive fields were identified in the peripheral lung by recording single-unit activities from the cervical vagus nerve using conventional methods [11, 12]. 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 caudal end of the vagus nerve, leaving the main trunk intact to continue to propagate afferent signals. This afferent bun-

Table 1. Categorization of SARs and their response to PEEP

SAR type	Deactivation	Nondeactivation	Total
High-threshold	1	10	11
Low-threshold	3	4	7
Total	4	14	18

High-threshold SARs are those discharged only during the lung inflation phase. Low-threshold SARs are those discharged continuously throughout the ventilatory cycle, i.e. during both the lung inflation and deflation phases. Although not statistically significant, the trend indicates that low-threshold SARs are more easily deactivated.

dle was dissected with two pairs of fine forceps into thin filaments, which were then separated into finer filaments and were placed on an electrode to record single-unit action potentials. The electrodes were connected to a high-impedance probe (model HIP511) from which the output was led to a Grass (P511) amplifier. After suitable amplification, the single-unit activities were displayed on an oscilloscope, and acoustically monitored by an attached loudspeaker. In addition, a voltage analog of impulse frequency was produced by a rate-meter (Frederick Haer, Brunswick, Maine, USA) at a bin width of 0.1 s.

Baseline unit activity was calculated for three ventilator cycles prior to raising PEEP in each rabbit, and served as its own control (measured in impulses per second, imp/s). Peak response was obtained over three ventilator cycles following the application of 10 cm H₂O PEEP after airway pressure reached maximum. The activity at the end of PEEP maneuver was calculated for the three ventilator cycles prior to reducing PEEP to 3 cm H₂O. Mean activity over three respiratory cycles as well as the maximum and minimum activity were determined for each single unit (expressed as imp/s).

Statistical Analysis

Data were analyzed by statistical software (STATA version 11, StataCorp LP, Tex., USA). Continuous variables were analyzed with the two-sample t test. A p value <0.05 was considered significant.

Results

Eighteen SARs were recorded, 7 low-threshold and 11 high-threshold SARs (table 1). Fourteen continuously discharged while the other 4 suddenly stopped their discharge during the 20-min 10 cm H₂O PEEP application. Figure 1 shows a typical SAR recording in 14/18 units (77%). The tracing begins with the rabbit ventilated at 3 cm H₂O PEEP for 4 breaths followed by elevation of the PEEP to 10 cm H₂O. This resulted in an immediate in-

Fig. 1. A typical SAR, recorded from the left cervical vagus nerve in an anesthetized, open-chest and mechanically ventilated rabbit. The tracings are unit activity expressed as imp/s, unit activity (Imp), and airway pressure (Paw). The rabbit was ventilated with a PEEP of 3 cm H₂O. **a** PEEP was then increased to 10 cm H₂O. Increasing PEEP from 3 to 10 cm H₂O significantly increased the airway pressure swing and the SAR discharged continually at the

beginning. The time lapse from **a** to **b** and **b** to **c** was 20 min and 1 min, respectively. At the end of the 20-min hyperinflation, the SAR discharged intermittently as a typical high-threshold SAR. **b** Peak activity was sustained. **c** Upon returning the PEEP to 3 cm H₂O, the SAR discharged significantly less than at baseline, and after 1 min, it had almost recovered. This represents 14/18 SARs tested.

Fig. 2. A typical SAR that deactivated during 30 cm H₂O constant pressure inflation and also during 10 cm H₂O PEEP application with cyclic ventilation. **a** Unit activity (Imp) ceased during lung deflation with a negative pressure. **b, c** Lung inflation at a constant positive pressure of 10 and 30 cm H₂O, respectively. **d** SAR activity during application of 10 cm H₂O PEEP (i.e. during intermittent positive pressure ventilation). The unit resumed its activity shortly after returning of the airway pressure (Paw) to the normal level. This represents 4/18 SARs tested.

crease in mean activity from 35.7 ± 4.2 to 80.5 ± 7.3 imp/s ($n = 14$, $p < 0.01$), reaching a 125% increase in discharge (table 2). However, over the course of 20 min of 10 cm H₂O PEEP, the activity decreased, reaching a low new level (56.5 ± 5.7 imp/s, $n = 14$, $p < 0.01$) that was still 58% above the baseline, a 30% decrease from the initial measurement after application of PEEP. Similarly, the maximum and minimum discharge activities were also increased by 62.8% ($p < 0.001$) and 462.4% ($p < 0.001$) initially following the 10 cm H₂O PEEP application. After 20 min, these maximum and minimum discharge rates were still elevated but decreased to 40.7 and 160% above the

baseline. Upon returning PEEP to 3 cm H₂O, the activity diminished below the control and then gradually recovered to the control level within 1 min (fig. 1).

There was different behavior in 4/18 units (22%), with an abrupt cessation of activity. Three were low-threshold (75%) and 1 was a high-threshold SAR (25%). They had an initial surge of receptor activity after 10 cm H₂O PEEP application similar to that seen in figure 1, which was followed by an abrupt termination of discharges (fig. 2) at a mean duration of 34.7 s (range 10–56 s). These deactivated units could be reactivated by lowering the inflation pressure and deactivated again by

Table 2. SAR activity in response to 10 cm H₂O PEEP application

	SAR activity, imp/s	Control	Start ^a of PEEP	End ^b of PEEP
Group 1 (n = 14)	Mean	35.7±4.2	80.5±7.3	56.5±5.7
	Maximum	84.2±5.2	137.1±11.4	118.5±9.3
	Minimum	8.5±3.7	47.8±6.2	22.1±4.9
Group 2 (n = 18)	Mean	35.5±3.5	86.2±7.1	44.0±7.2
	Maximum	82.2±4.4	142.2±9.5	92.2±13.9
	Minimum	7.2±3.0	52.2±6.1	17.2±4.4

Mean activity was averaged over three respiratory cycles; maximum (minimum) activity is the highest (lowest) discharge frequency during 0.1 s within a cycle. The differences are significant between baseline and the start of PEEP ($p < 0.01$) and between the start of PEEP and the end of PEEP ($p < 0.01$) in both groups. Group 1: deactivated SARs removed. Group 2: all SARs.

^a At the fifth ventilator cycle of the application of PEEP. ^b At the fifth ventilator cycle before ending the PEEP application.

Table 3. Peak airway pressure during 10 cm H₂O PEEP application

	Control	Start ^a of PEEP	End ^b of PEEP
Mean, cm H ₂ O	9.3	32.7	29.4
SD, cm H ₂ O	1.3	7.6	5.1
SE, cm H ₂ O	0.31	1.8	1.2

The difference in peak airway pressure is statistically significant (n = 18; $p < 0.01$ between control and start of PEEP; $p < 0.05$ between start and end of PEEP).

^{a, b} Same as in table 2.

returning to the high pressure. Activation and deactivation occurred abruptly, with unit activity oscillating between high frequencies and zero. These 4 units became deactivated at a constant pressure inflation of 30 cm H₂O but not at 10 cm H₂O (fig. 2).

During the course of 10 cm H₂O PEEP application, peak airway pressure increased initially, and then decreased gradually. Although this change was small (3.3 cm H₂O), it was statistically significant (fig. 1; table 3).

Discussion

Our data demonstrate a dichotomous SAR response during 20 min of 10 cm H₂O PEEP application. Four SARs deactivated after applying PEEP; in 14, the activity

increased initially and then declined gradually to a steady state above the baseline. In the meantime, the airway pressure also decreased to an extent that was small but statistically significant. This suggests multiple mechanisms including receptor deactivation, mechanical adaptation and neural habituation for downregulating SAR activity during continuous PEEP.

Deactivation of SARs has been reported following lung hyperinflation under a constant pressure in rabbits [13], cats [14] and opossums [15]. Deactivation also occurs after injecting ouabain, an inhibitor of Na⁺/K⁺-ATPase, into a vein [16] or directly into the receptive field [17]. Our study demonstrates that SARs can be deactivated under high cyclic airway pressure (induced by PEEP). It is possible that deactivation is due to a deficiency of Na⁺/K⁺-ATPase from metabolic stresses or depletion [13]. The majority (75%) of the deactivated SARs were low-threshold SARs. This is consistent with previous reports that SARs with a high discharge frequency are more susceptible to deactivation [13, 15]. Since low-threshold SARs fire continuously throughout the respiratory phase [1], they are more metabolically active. Similarly, some arterial baroreceptors are stimulated and then deactivated after bolus injection of phenylephrine to increase blood pressure [18]. This mechanism of deactivation thus seems to be universal across mechanosensors.

The activity of the 4 deactivated units ceased shortly after applying 30 cm H₂O constant pressure but not with 10 cm H₂O (fig. 2). This implies that the increased airway pressure secondary to PEEP, but not the PEEP itself, was responsible for SAR deactivation. Therefore, airway pressure is an important determinant of the SAR behavior. Airway pressure declined during high PEEP application (fig. 1; table 3). This may result from recruitment of previously collapsed lung units [7], causing air redistribution and pressure reduction. Such pressure-reduction-induced decrease in SAR activity is referred to as mechanical adaptation [19].

Mechanosensors, including SARs, adapt during sustained simulation [1, 3, 19, 20]. This is called neural habituation. At present, we do not know its underlying mechanisms. DiCaprio et al. [21] demonstrated an activity-related component of afferents in the Golgi tendon organs of insects that occurs with either electrical or mechanical stimulation. They believed that Ca²⁺-dependent K⁺ channels generated slowly after hyperpolarization, restricting the generation of action potentials as previously suggested by Erxleben [22]. They added Ba²⁺ to the solution surrounding tendon organs, thus preventing the influx of Ca²⁺, and blocked the adaptation of these mecha-

nosensors. They also demonstrated that high levels of Ca^{2+} exaggerated adaptation [21].

In this study, we determined the relative importance of the three mechanisms in reducing SAR activity during PEEP application. Clearly, receptor deactivation and neural habituation are major mechanisms while mechanical adaptation plays a minor role in decreased SAR activity. When all the units were included ($n = 18$), SAR activity decreased from 86.2 to 44 imp/s during the 20-min PEEP application. Removing the 4 deactivated units ($n = 14$), SAR activity decreased from 80.5 to 56.5 imp/s. The difference between the two resultant values, i.e. $42.2 - 24 = 18.2$ imp/s was caused by receptor deactivation, accounting for 43.1% ($18.2 \div 42.2 \times 100$) of the total SAR decrease during the PEEP application. Therefore, neural habituation and mechanical adaptation together accounted for 56.9% (i.e. $100 - 43.1$). The application of 10 cm H_2O PEEP increased peak airway pressure from 9.3 to 32.7 cm H_2O and receptor activity from 35.7 to 80.5 imp/s. Assuming a linear relationship, this gives a 1.9-imp/s increase per cm H_2O . For a 3.3-cm H_2O pressure difference between the start and end of PEEP application, the receptor activity would decrease by 6.3 imp/s. Since neural habituation and mechanical adaptation accounted for 56.9% of the total decrease in SAR activity, mechanical adaptation would account for 14.9% [$6.3 \div (80.5 - 56.5) \times 56.9\%$] and neural habituation for 42%. In fact, total SAR input in relation to the airway pressure is nonlinear. Many SARs do not increase as much at high pressure, thus mechanical adaptation should be $<14.9\%$.

The SAR is stimulated when airways where the receptor is located are stretched. The SAR activity is determined not only by external stimuli (stretch of the airway by pressure), but also by its internal properties (e.g. its sensitivity to stretch and threshold for activation) [3]. An increased PEEP (as CPAP application in the clinical setting) would, of course, simultaneously increase airway pressure, thereby stimulating the SAR. The stimulus can be the peak pressure, the integrated pressure, the pressure profile or a combination of these. We believe that stretch of the airway will activate the SAR and increase its metabolism. In our previous studies, we found the more active the SARs, the easier they were to deactivate [13]. Furthermore, lung inflation-induced SAR deactivation was more closely associated with receptor activity than the airway pressure. Priming the airway with a lower level of constant pressure (like PEEP application) keeps the SAR active and promotes its deactivation [13].

It is desirable to apply the knowledge obtained from animal studies to assist clinical practice. Haxhiu et al.

[10] demonstrated that after 1 min of lung inflation by PEEP, the expiratory time gradually returns to baseline values. Such a progressive diminution of response can be explained by the gradual decline of SAR activity. Distention of the airway initially leads to increased SAR signals, resulting in decreased phrenic nerve activity [9] and, in turn, prolonged expiration [10]. In COPD patients, the HBR was weakened [8]. Possibly, the intrinsic PEEP present for years in COPD patients leads to an attenuation of SAR responses to elevated pressures. The HBR can be a protective mechanism to reduce lung overdistention and resultant volutrauma. There are numerous iatrogenic (e.g. mechanical ventilation) and pathological (e.g. COPD) processes that distend the lungs; SARs play a role here. With prolonged stimulation, SAR activity gradually returns toward baseline, blunting the reflex response and leading to a relatively normal inspiratory and expiratory time. This mechanism may explain why COPD patients have a minimally prolonged expiratory time. Waters et al. [23] demonstrated that 25% of children receiving CPAP titration developed hypoventilation at high pressures, a finding long observed in both pediatric and adult patients. Excessive BPAP or CPAP in obstructive sleep apnea therapy could actually increase apneic episodes, counteracting treatment benefits. Some patients with apparent obstructive sleep apnea-hypopnea syndrome experience an elimination of obstructive events but an emergence of central apnea after exposure to CPAP or BPAP [24], i.e. the so-called complex sleep apnea syndrome. Fortunately, a decreased SAR input diminishes the respiratory inhibition and prevents potential hypoxia.

It is also important to consider the impact of the HBR on ICU patients who are being weaned by means of pressure support. The rapid shallow breathing index ($\text{RSBI} = f/V_t$; respiratory rate in breaths/min divided by tidal volume in liters) is often used to predict successful extubation. High airway pressure could prolong expiratory time and decrease respiratory frequency, creating a misleading and false sense of security for extubating. Since high airway pressures may cause apneic episodes through the HBR, the question is raised whether patients with compromised pulmonary function would be better-served by a gradual application of positive pressure support similar to that applied during sleep-laboratory titration as opposed to a rapid application. If, indeed, time should be allowed for adaptation, as demonstrated in our study, this could have important ramifications for the application of noninvasive mechanical ventilation; this needs to be followed up in clinical trials.

One of the limitations of this study is our use of an animal model. Although this was necessary for studying the underlying mechanisms, it means that a direct translation of the results to humans is not feasible. In addition, we employed open-chest preparation. This differs from the closed-chest procedure in that it exposes the lung directly to the atmosphere, which may affect the SAR behavior. However, several investigators show similar receptor activities in open- versus closed-chest preparations [25–27]. Since the difference was quantitative but not qualitative, our study did provide potential mechanisms of SAR behavior during PEEP application.

Conclusions

SARs may be deactivated under high-pressure, cyclic ventilation. High PEEP vigorously stimulated SARs, but the total SAR input gradually returned towards the baseline via multiple mechanisms including receptor deactivation, neural habituation and mechanical adaptation.

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No conflict of interest exists for any authors.

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