Influence of acute lung volume change on contractile properties of human diaphragm

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Influence of acute lung volume change on contractile properties of human diaphragm. J. Appl. Physiol. 85(4): 1322–1328, 1998.—The effect of stimulus frequency on the in vivo pressure generating capacity of the human diaphragm is unknown at lung volumes other than functional residual capacity. The transdiaphragmatic pressure (Pdi) produced by a pair of phrenic nerve stimuli may be viewed as the sum of the Pdi elicited by the first (T1 Pdi) and second (T2 Pdi) stimuli. We used bilateral anterior supramaximal magnetic phrenic nerve stimulation and a digital subtraction technique to obtain the T2 Pdi at interstimulus intervals of 999, 100, 50, 33, and 10 ms in eight normal subjects at lung volumes between residual volume and total lung capacity. The reduction in T2 Pdi that we observed as lung volume increased was greatest at long interstimulus intervals, whereas the T2 Pdi obtained with short interstimulus intervals remained relatively stable over the 50% of vital capacity around functional residual capacity. For all interstimulus intervals, the total pressure produced by the pair decreased as a function of increasing lung volume. These data demonstrate that, in the human diaphragm, hyperinflation has a disproportionately severe effect on the summation of pressure responses elicited by low-frequency stimulations; this effect is distinct from and additional to the known length-tension relationship.

THE KNOWN EFFECTS of acute lung volume change on the contractile properties of the in vivo human diaphragm have been obtained by measuring transdiaphragmatic pressure (Pdi) in conjunction with a maximal voluntary effort (1, 41), or single bilateral supramaximal phrenic nerve stimulation (20, 38), or both (27, 37). Although these studies show, as expected, that hyperinflation results in reduction in the pressure-generating capacity of the diaphragm, they do not address the effect of stimulus frequency on pressure generation. Thus the relevance of these data to the acutely hyperinflated patient, who is required to generate a Pdi for hours or days, depends crucially on how representative these techniques are of sustainable in vivo phrenic nerve firing rates.

Although few data are available, motor unit discharge rates for both the diaphragm and other inspiratory muscles in normal subjects at functional residual capacity (FRC) have been reported ~10 Hz (8, 13), whereas patients with very severe chronic obstructive pulmonary disease (COPD) have resting diaphragm motor unit discharge rates between 10 and 20 Hz (8). Diaphragm motor unit discharge rates have not been recorded during a maximal inspiratory maneuver in humans, but, during a maximal voluntary contraction (MVC) of limb muscle, peak discharge rates may be 50 Hz or greater (16, 21). Thus Pdi measurements obtained from either a maximal voluntary effort or from single twitches may be unrepresentative of in vivo diaphragm performance.

That single twitches and MVCs do not reflect in vivo firing rates would be of only theoretical interest if change in muscle length influenced the entire force-frequency curve in equal proportions. In fact, for limb muscle, there are convincing data both in humans (14, 26) and animals (34) that this is not so; this phenomenon is sometimes termed the length dependence of activation (LDA). Therefore, the hypothesis of the present investigation was that lung volume change does not result in an equal diminution of Pdi in response to all stimulation frequencies and that, as in limb muscle, the reduction in pressure generation is greatest at low frequencies.

Tetanic supramaximal bilateral electrical stimulation (ES) has not proved feasible at lung volumes other than relaxed end expiration. Therefore, we considered this technique unsuitable to test our hypothesis. The Pdi generated by two stimuli may be considered the sum of the Pdi produced by the first stimulus (T1 Pdi) and the second stimulus (T2 Pdi). The relationship between interstimulus interval and the T2 Pdi (the T2 force-frequency relationship) is a measure of the capacity of twitches to summate (7, 9, 19), and, at least for the detection of qualitative changes, this approach could therefore be an alternative to the construction of a formal force-frequency curve. This technique has recently been successfully applied to the human diaphragm in vivo (44). Thus, if our hypothesis were correct, the shape of the T2 force-frequency relationship would be influenced by lung volume. If our hypothesis were incorrect, the shape of the T2 force-frequency relationship would be the same over a range of lung volumes.

METHODS

Data Acquisition

Pdi was obtained by using a pair of commercially available latex balloon catheters (PK Morgan, Rainham, Kent, UK) 110 cm in length; these were placed in the stomach and esophagus in the conventional manner. The catheters were connected to...
diaphragm contractility in acute hyperinflation

Subjects

The subjects were eight healthy members of the laboratory staff (7 men and 1 woman) who were free of neurological and respiratory disease. The protocol was approved by the institutional ethical committee of King's College School of Medicine and Dentistry, and all subjects gave informed consent to participate.

Lung Volume Measurement

Inspired and expired volume changes were measured by a spirometer (Ohio 840; Airco, Houston, TX) connected to a flanged mouthpiece via a closed circuit.

Phrenic Nerve Stimulation

The phrenic nerves were stimulated by using simultaneous bilateral anterior magnetic stimulation of the phrenic nerves (BAMPS; see Ref. 28). Paired stimuli were given from two 40-mm figure-eight coils, each of which was powered by two Magstim 200 stimulators (Magstim, Whitland, Dyfed, UK). Each pair of stimulators was linked by circuitry (BiStim Module, Magstim) that was capable of precisely controlling the interstimulus interval between 1 and 999 ms, to an accuracy of within 0.05 ms.

Protocol

At the start of the study, the vital capacity (VC) and inspiratory capacity (IC) for each subject were determined. After a 20-min rest period (to minimize twitch potentiation (23, 43)), the exact site for optimal phrenic nerve stimulation was determined and marked. In each subject, a series of single bilateral phrenic nerve stimuli at varying power outputs was then administered to assess the interstimulus interval between 1 and 999 ms, to an accuracy of within 0.05 ms.

Data Analysis

Twitches were only accepted for analysis if performed with the subject relaxed, as judged by Pes, and when baseline Pdi was similar to that seen at end expiration during normal breathing, indicating relaxation of the diaphragm. Twitches of Pes, Pga, and Pdi (Pdi_{tw}, Pga_{tw}, and Pdi_{ptw}, respectively) were defined as the difference between peak pressure immediately after stimulation and the baseline pressures immediately before. The pressure addition caused by the second stimulus (T2 Pdi) was obtained by using a modification of LabView software that permitted digital subtraction of a single twitch from the pair (30) in the manner described by Yan and colleagues (44). An example is shown in Fig. 1. The purpose of the software modification was to allow the superimposition of curves obtained at each given experimental condition to generate an averaged signal. To allow accurate superimposition, we aligned the signals temporally by using the marker signal produced by the discharge of the first pair of magnets. To obtain the T2 signal, the averaged response to a single stimulus was subtracted from the averaged response to a paired stimulation at 10-ms intervals (reflecting the sampling frequency of 100 Hz). Once the T2 signal was obtained, the amplitude was calculated by measurement from baseline to peak (Fig. 1). Statistics were computed by using Statview 4.0 (Abacus Concepts, Berkeley, CA) and simple or polynomial regression analysis.

Conventions

The pressure response to a single bilateral twitch is denoted by Pdi_{tw}, and to a paired twitch by T2 Pdi_{ptw}. To facilitate discussion, we have sometimes used stimulation frequency (in Hz) as a shorthand for the reciprocal of the interstimulus interval (in ms); however, on no occasion were sequences longer than two stimuli used.

RESULTS

Stimulation was tolerable in all subjects. Pdi responses at FRC are shown in Table 1. The Pdi obtained

![Fig. 1. Representative traces obtained during paired bilateral anterior magnetic phrenic nerve stimulation with an interstimulus interval of 100 ms (10 Hz). Each line is averaged response of a minimum of 3 stimulations. Subtraction of twitch transdiaphragmatic pressure (Pdi_{tw}) from paired Pdi_{tw} (Pdi_{ptw}) yields second stimulus in isolation (T2 Pdi). This may be expressed as a function of interstimulus interval (or frequency) to give a T2 force-frequency curve.](image-url)
is upward sloping, such that the T2 Pdi at 10 Hz is greater than that obtained at 30 or 100 Hz. During acute hyperinflation, this pattern is reversed such that the T2 Pdi at 10 Hz is smaller than that obtained at 30 and 100 Hz. This change in the shape of the T2 force-frequency relationship moving from RV to TLC is progressive. The T2 Pdi for interstimulus intervals of 100 ms (10 Hz) and 10 ms (100 Hz) was expressed as a function of lung volume (Fig. 6). The 20- and 30-Hz curves are omitted for clarity, but they lay between these two curves. This plot shows that the 10-Hz T2 Pdi is reduced at high lung volume and increased at RV; in contrast, the 100-Hz T2 Pdi at short interstimulus intervals does not decline appreciably until lung volume is >50% of VC. Expressing the T2 responses numerically, following Yan and co-workers (44), as the ratio of the 10-Hz to the 100-Hz T2 Pdi, or the T2<sub>10:100</sub>, there was a negative correlation (r² = 0.93, P < 0.001) of this value with increasing lung volume.

**DISCUSSION**

Our data support the hypothesis that change in lung volume has an unequal effect on the pressure generated by the diaphragm in response to different phrenic nerve stimulation frequencies. This indicates that, like limb muscle, the diaphragm exhibits LDA. Before consideration of the significance of this observation, methodological considerations will be addressed.

Critique of the Methods

Use of the T2 force-frequency relationship. The use of the T2 force-frequency relationship is subject to two main criticisms: 1) it would have been preferable to use tetanic stimuli, and 2) the T2 relationship is not sufficiently robust.

When percutaneous bilateral electrical stimulation (ES) is used, accurate focusing of the electrodes is crucial, and, during tetanic ES, it would be important to maintain electrode position, which is technically very difficult. Reported studies that used tetanic ES at FRC in experienced subjects have been limited by not using it bilaterally (2, 29) or not using the full range of stimulation frequencies (4). For these technical reasons, it is unlikely that the influence of lung volume change on the force-frequency relationship of the diaphragm could be studied in vivo by using tetanic ES.

Paired nerve stimuli are an established physiological technique (7, 19). In particular, the contractile properties of both whole muscle and individual motor units are similar, whether they are assessed by tetanic or paired stimuli (9). Paired ES (pES) was recently applied to the phrenic nerves by Yan and co-workers (44), who also used digital subtraction techniques to isolate the influence of interstimulus interval on the pressure addition caused by the second stimulus. They showed that the technique was capable of detecting changes in the force-frequency relationship (after the induction of low-frequency fatigue) in both the in vitro rat and in vivo human diaphragm. Using paired cervical magnetic stimulation (pCMS), we also recently showed that a
protocol (2 min of maximal isocapnic ventilation) known to induce diaphragm fatigue (18, 28) also produced a reduction in the T2 Pdi at long interstimulus intervals (31). These studies suggest that T2 analysis of the Pdi generated by paired stimuli can indeed detect changes in the contractile properties of the diaphragm, although it is more difficult to qualitatively predict the changes that would be observed in the tetanic force-frequency curve from the T2 data.

Diaphragm length and configuration. Yan and colleagues (44) suggest that, when paired stimuli are used, it is preferable to bind the abdomen. Their reasoning was, presumably, based on the need to keep diaphragm configuration constant throughout both twitches. However, we opted not to bind the abdomen because lung volume does not influence the displacement of the rib cage and abdomen produced by phrenic nerve stimulation with an unbound abdomen (20), and thus there is no reason to suspect that geometrical considerations prevented accurate recording of the influence of lung volume on the pressure generated by the diaphragm. The reasoning of Yan et al. was based on two papers (3, 42) which, like our own (22), show that binding increases the numerical value of Pga\text{tw} (and hence Pdi\text{tw}), presumably by stiffening the abdominal wall. Importantly, none of these studies found binding to significantly reduce variation in the value of Pdi\text{tw} (3, 22, 42). Moreover, the relaxation time (which clearly influences the T2 force-frequency relationship) is not changed by abdominal binding (42). Conversely, magnetic stimulation techniques (whether by the cervical or bilateral anterior approach) do seem to be less variable than ES (28).

Use of BAMPS. The paired stimuli could have been generated by pES (44), pCMS (30, 31), or paired BAMPS (pBAMPS). We did not use pES because of concern that, at lung volumes other than FRC, the technical difficulty of maintaining electrode position would have necessitated the administration of additional stimuli (to refocus electrode position) making it difficult to avoid twitch potentiation (43). We have previously demonstrated that this problem can influence the results obtained with paired nerve stimuli both of the phrenic nerves (23) and the thoracic nerve roots (24). The problem of potentiation was also identified as a difficulty with pES by Yan and co-workers (44). pCMS minimizes the problem of twitch potentiation, but we chose not to use the technique for this study because of the difficulty in demonstrating supramaximality of stimulus intensity (25, 36). pBAMPS has the advantage of minimizing twitch potentiation but, in addition, is also selective for the diaphragm (28), allowing the investigator to be confident of supramaximality by assessment of the Pdi\text{tw}. Nevertheless, the conclusion of the present study was anticipated from a
previous study in which we examined the effect of a limited lung volume change by using pCMS (31), which suggested that it might have been possible to make similar observations by using pCMS.

**Significance of the Findings**

Our study confirms that increasing lung volume reduces the pressure-generating capacity of the diaphragm over the range of interstimulus intervals between 10 and 999 ms. For the unpotentiated Pdi low, the magnitude of change (0.3 cmH₂O/%VC) was very similar to that previously reported by us with CMS (17). We further show that acute diaphragm shortening has the greatest effect on the summation of twitches generated by low-frequency stimulation. Thus the human diaphragm in vivo exhibits the property of LDA, which is recognized in skeletal muscle. This phenomenon has been reported from studies of isolated mammalian limb (34) and diaphragm muscle (11), as well as in vivo studies of human limb muscle (14). The functional consequence of LDA is that, at least for isometric contractions, proportionately higher stimulation frequencies are required to maintain tension generation at short muscle lengths. Although not addressed in the present study, the mechanisms underlying LDA are of interest. Muscle contraction occurs as a result of intracellular calcium release from the sarcoplasmic reticulum. Low-frequency stimulation results in fewer action potentials. However, for this to result in a disproportionate force loss at short muscle length would require that, at short lengths, either the contractile elements are less sensitive to the calcium released from the sarcoplasmic reticulum or that less calcium is released. Some investigators have proposed that the latter occurs as a consequence of impaired action potential propagation within the t tubule system. However, data reviewed by Stephenson and Wendt (39) showed, in skinned fibers, that the optimal sarcomere length increases as calcium level falls. This effect was consider by Roszek et al. (35) to be of sufficient magnitude to explain LDA observed in a rat gastrocnemius preparation.

Evanich and co-workers (10) demonstrated in the in vivo feline diaphragm that, as in the present study, the diaphragm length-tension relationship is predominantly mediated by changes in the esophageal component of the Pdi. They also demonstrated the presence of LDA. Thus our data are consistent with previously reported data; nevertheless, the combined effects of length and stimulus frequency on human diaphragm pressure-generating capacity have not been previously studied in vivo. In this respect, the data from Fig. 6 are of interest because they suggest that length change around FRC might exert its greatest effect via LDA, whereas at higher lung volumes the length-tension relationship might be of greater importance.

During acute hyperinflation, normal subjects maintain tidal volume by increasing the (electrical) activity of the inspiratory muscles (15). This would be expected as a result of length tension and does not, in itself, demonstrate LDA. Our data do demonstrate LDA and suggest that patients who are acutely hyperinflated must increase phrenic nerve activity (by increasing either discharge frequency or recruitment) more than would be expected simply as a result of length tension, unless Pdi generation is sacrificed. It is likely that the diaphragm does make a reduced contribution to ventilation during acute hyperinflation in both normal subjects (6, 45) and patients with COPD (33). However studies demonstrating reduced diaphragm pressure generation do not distinguish between a reduced contribution as a result simply of the length-tension relationship or because of the phenomenon of LDA as demonstrated by our data.

Diaphragm motor unit discharge rates are increased in patients with chronic hyperinflation at rest (8), but, to our knowledge, no studies have investigated the influence of acute hyperinflation on motor unit dis-
charge rates directly for the human diaphragm. However, data from studies in the in vivo human tibialis may provide some insight. Bigland-Ritchie and colleagues (5) examined contractions between 50 and 100% of the MVC force. They found that discharge rates over this range of intensities of voluntary contraction were independent of length. The proposed explanation was that, for submaximal contractions in this range, additional motor units are recruited to compensate for the reduced tension generated by those already recruited. However, for weaker contractions (up to 40% of MVC), it has been shown that the motor unit discharge rate is, as expected, greater at short muscle lengths (40).

During exercise in COPD, when acute dynamic hyperinflation occurs, it is likely that phrenic nerve firing rates are greatly elevated in an attempt to maintain Pdi generation. Because respiratory load estimation is related to the size of motor command, rather than to the load (12), we speculate that the mechanisms demonstrated by our data might contribute to the sensation of dyspnea in this situation.

An expiratory deflection of Pesaw after bilateral ES is a recognized, but occasional, observation in normal subjects at TLC (25, 37, 38); however, it is relatively common after cervical magnetic stimulation (CMS) of the phrenic nerve roots in normal subjects (17, 25). This phenomenon has also been observed in patients with extreme chronic hyperinflation after CMS (32) but not ES (37). Laghi et al. (25) proposed that this occurred because of coactivation of upper thoracic expiratory muscles. BAMPS is considered to be more selective than CMS for the diaphragm (28); nevertheless, in six of eight subjects in the present study, an expiratory deflection of Pes was observed at TLC. These data are consistent with the hypothesis that, like CMS, BAMPS also causes relevant coactivation of expiratory upper thoracic muscles. Alternatively, the data support the notion that the diaphragm can be expiratory at high lung volume and that this is demonstrable, because achieving reliable phrenic nerve stimulation at TLC is technically easier with BAMPS than with ES.

In conclusion, our data show that, like other skeletal muscles, the pressure-generating capacity of the human diaphragm in vivo is influenced by the frequency of stimulation; specifically, shortening disproportionately reduces the force response to low-frequency stimulation, typical of those encountered in life. Further studies are warranted to examine discharge frequencies and motor unit recruitment patterns of the human diaphragm during voluntary contractions at short muscle length.

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