Effect of inflation on the interaction between the left and right hemidiaphragms

André De Troyer, Matteo Cappello, and Pierre Scillia

Laboratory of Cardiorespiratory Physiology, Brussels School of Medicine, and Department of Radiology, Erasme University Hospital, Brussels, Belgium

Submitted 16 February 2005; accepted in final form 6 May 2005

De Troyer, André, Matteo Cappello, and Pierre Scillia. Effect of inflation on the interaction between the left and right hemidiaphragms. J Appl Physiol 99: 1301–1307, 2005.—At resting end expiration [functional residual capacity (FRC)], the actions of the left and right hemidiaphragms on the lung are synergistic. However, the synergism decreases in magnitude as muscle tension decreases. Therefore, the hypothesis was tested in anesthetized dogs that the degree of synergism between the two hemidiaphragms also decreases with increasing lung volume. In a first experiment, the changes in airway opening pressure (ΔPao) and abdominal pressure (ΔPab) obtained during simultaneous stimulation of the left and right phrenic nerves (measured changes in pressure) at different lung volumes were compared with the sum of the pressure changes produced by their separate stimulation (predicted changes in pressure). Although the pressure changes decreased markedly with increasing lung volume, the measured ΔPao and ΔPab were substantially greater than the predicted values at all lung volumes. The ratio of the measured to the predicted ΔPao, in fact, remained constant. In a second experiment, radiographic measurements showed that the fractional shortening of the muscle during bilateral contraction at high lung volumes was similar to that during unilateral contraction. During unilateral contraction at high lung volumes, however, the passive hemidiaphragm moved in the cranial direction, whereas, during unilateral contraction at FRC, it moved in the caudal direction. These observations indicate that 1) for a given muscle tension, the synergism between the two halves of the diaphragm is greater at high lung volumes than at FRC; and 2) this difference is primarily related to the greater distortion of the muscle configuration.

The changes in intrathoracic pressure generated by the various inspiratory intercostal muscles are essentially additive. Thus, in the dog with the airway occluded at resting end expiration [functional residual capacity (FRC)], the change in airway opening pressure (ΔPao) produced by the simultaneous, bilateral contraction of the parasternal intercostals or external intercostals in two interspaces is, within 10%, equal to the sum of the ΔPao values produced by bilateral contraction of the muscles in each individual interspace (10). The ΔPao produced by the simultaneous contraction of the parasternal or external intercostals in one or two interspaces on the left and right sides of the sternum is also nearly equal to the sum of the ΔPao values produced by separate left and right muscle contractions (3). On the other hand, when the phrenic nerves in dogs (4) and in humans (1) are selectively stimulated at FRC, the ΔPao obtained during bilateral stimulation is clearly greater than the sum of the ΔPao values obtained during separate left and right stimulation, thus indicating that the actions of the left and right halves of the diaphragm on the lung are synergistic, rather than additive. This difference between the two halves of the diaphragm and the intercostal muscles is primarily related to the fact that the diaphragm has less constraint on its configuration than the rib cage (4).

The magnitude of the synergism between the two hemidiaphragms, however, is closely related to the tension developed by the muscle fibers, as shown in Fig. 1. The data in this figure are those obtained for all animals and all frequencies of phrenic nerve stimulation in our previous study (4), but here these data are replotted with pressure as the independent variable rather than stimulation frequency. That is, the sums of the ΔPao values obtained during separate left and right stimulations are plotted along the abscissa (for the sake of consistency with our laboratory’s previous studies, Refs. 3, 4, 10, these sums will be referred to here as the predicted values), and the values of ΔPao measured during simultaneous left and right stimulation are plotted along the ordinate. Although all values of ΔPao during bilateral stimulation are greater than the predicted values, it can be seen that the ratio of the measured ΔPao to the predicted value decreases gradually as the predicted ΔPao decreases. For a predicted ΔPao of −30 to −50 cmH2O, the measured ΔPao is −50% greater than predicted, whereas, for a predicted ΔPao of −10 cmH2O, the measured ΔPao is hardly 10% greater than predicted. Thus, when muscle tension in the diaphragm is low, the actions of the two halves of the muscle on the lung are also essentially additive.

Inflating the respiratory system above FRC causes a marked shortening of the diaphragm, and, hence, the tension developed by the muscle in response to a given activation also decreases (6, 8, 11, 14, 15). In the present study, therefore, the hypothesis was tested that the degree of synergism between the two halves of the diaphragm similarly decreases with increasing lung volume. This hypothesis, however, was not confirmed, and the degree of synergism was found to be independent of lung volume. In a second experiment, therefore, radiographic measurements of the changes in diaphragmatic muscle length and configuration during bilateral and unilateral phrenic nerve stimulation at high lung volumes were also performed to assess the mechanism of this phenomenon.

METHODS

The studies were carried out on 11 adult mongrel dogs (body wt: 13–26 kg) anesthetized with pentobarbital sodium (initial dose: 30

Address for reprint requests and other correspondence: A. De Troyer, Chest Service, Erasme Univ. Hospital, Route de Lennik, 808, 1070 Brussels, Belgium (e-mail: a.detroyer@yahoo.fr).

http://www.jap.org 8750-7587/05 $8.00 Copyright © 2005 the American Physiological Society 1301
was then reconnected to the ventilator and hyperventilated, and the impulses/s for 2–3 s to the left C5 and C6 phrenic nerves. The animal duration and supramaximal voltage were applied at a frequency of 50 Hz. The endotracheal tube was occluded at FRC, and square pulses of 0.1-ms duration and supramaximal voltage were applied at a frequency of 50 Hz. The animal was returned to the assisted ventilation, after which the ventilation was stopped, the stimulating electrodes.

Experiment 1. Four animals were subsequently studied to examine the changes in length of the diaphragm and the alterations in diaphragmatic silhouette during unilateral vs. bilateral contraction at high lung volumes. The procedure was similar to that previously described (4, 16). Thus, in each animal, the abdomen was opened by a midline incision from the xiphisternum to the umbilicus, and rows of five lead spheres (diameter: 4–5 mm) were stitched to the peritoneal surface and superficial muscle fibers of the left and right hemidiaphragms in the coronal midplane. Typically, the markers attached to the cranial half of the muscle were spaced at ~15- to 20-mm intervals, and those attached to the caudal half, in the zone of apposition of the diaphragm to the rib cage, were spaced at ~25- to 30-mm intervals. Consequently, the chord length between the successive markers closely approximated the arc length along the diaphragm. After the abdomen was closely sutured, the animal was placed supine in a radiolucent fabric sling and made apneic by mechanical hyperventilation, and anteroposterior radiographs of the lower rib cage and upper abdomen were taken first during relaxation at FRC, then during separate, supramaximal stimulation (50 Hz) of the left and right phrenic nerves, and finally during simultaneous stimulation of the left and right phrenic nerves. Lung volume was subsequently increased passively to a transrespiratory pressure of 20 cmH2O, and the procedure was repeated. As was the case in experiment 1, all stimulations were performed while the endotracheal tube was occluded.

The animals in both experiments were maintained at a constant, rather deep level of anesthesia throughout. They had no pupillary light reflex, no corneal reflex, and no movements of the fore- or hindlimbs, including during phrenic nerve stimulation. Rectal temperature was maintained constant between 36 and 38°C with infrared lamps. At the end of the study, the animal was given an overdose of anesthetic (30–40 mg/kg iv).

Data analysis. For each lung volume in each individual animal, the ΔPao and ΔPab values obtained during unilateral and bilateral stimulation of the C5-C6 phrenic nerves (experiment 1) were averaged over the three trials, and the pressures obtained during isolated left stimulation were added to the pressures obtained during isolated right stimulation to yield the “predicted” values. The pressures measured during bilateral stimulation at the different lung volumes and the predicted values were then plotted against the value of Pao before stimulation (i.e., the precontractile transrespiratory pressure), and the relationships were fitted by regression equations of the type $y = ae^{bx}$, where $y$ was the measured or the predicted pressure change value, and $x$ was the transrespiratory pressure; in each animal, the coefficient of correlation ($r$) of these relationships was ≥0.982. Measured and predicted ΔPao values and ΔPab values at fixed transrespiratory pressures at 5.0-cmH2O increments were determined from these equations by interpolation.

The changes in diaphragmatic muscle length induced by unilateral and bilateral phrenic nerve stimulation at FRC and at 20-cmH2O transrespiratory pressure (experiment 2) were first quantified by measuring the linear distance between adjacent radiopaque markers and by summing the distances between markers in each row. To allow comparison between the different animals, the changes in muscle length during stimulation at a given lung volume were then expressed as percentage changes relative to the muscle length during relaxation at this volume. In addition, the changes in diaphragmatic shape were examined by tracing the contour of the diaphragm in each condition and by superimposing the contours during unilateral and bilateral stimulation on that during relaxation. All contours were related to a metallic marker that was attached to the sling on the side of the animal and was, therefore, stationary. To quantify the changes in shape, the...
axial (craniocaudal) displacement of the dome in the two sagittal planes situated midway between the spinous processes of the vertebrae and the lateral rib cage margins was also measured. Because isolated stimulation of the right or the left phrenic nerve produced identical changes in muscle length and identical changes in shape, these changes were averaged for each individual animal.

Data of pressure, muscle length, and muscle shape were finally averaged across the animal group, and they are presented as means ± SE. Statistical comparison between the measured and predicted ΔPao and ΔPab values at the different transrespiratory pressures was made by ANOVA with repeated measures, and multiple-comparison testing of the mean values was performed by using Student-Newman-Keuls tests. Statistical comparisons between the changes in muscle length and the axial displacements of the dome during unilateral stimulation at FRC and 20-cmH₂O transrespiratory pressure were made by using paired t-tests. The criterion for statistical significance was taken as P < 0.05.

RESULTS

Pressures. The values of predicted and measured ΔPao obtained at the different lung volumes in the seven animals studied are shown in Fig. 2A. The measured ΔPao was substantially greater than the predicted value at all lung volumes (P < 0.001). In fact, although both the measured and the predicted ΔPao decreased markedly with increasing lung volume, the ratio of the measured over the predicted value remained constant (Fig. 2B); this ratio was, on average, 1.57 ± 0.06 at FRC and 1.55 ± 0.15 at 30-cmH₂O transrespiratory pressure. The measured ΔPab was also invariably greater (P < 0.001) than the predicted value, as shown in Fig. 3.

Length and shape of the diaphragm. The changes in diaphragmatic muscle length measured during bilateral and unilateral phrenic nerve stimulation at FRC and 20-cmH₂O transrespiratory pressure in the four individual animals are summarized in Table 1, and the changes in diaphragmatic silhouette are reproduced for a representative animal in Fig. 4. As was the case in experiment 1, the measured ΔPao was greater than the predicted value at both lung volumes in every animal (Table 1). Also, in agreement with our previous observations (4, 16), bilateral stimulation at both lung volumes caused a large shortening and a large caudal displacement of the two hemidiaphragms, and unilateral stimulation at both lung volumes caused a large shortening and a marked caudal displacement of the ipsilateral hemidiaphragm associated with a lengthening of the contralateral (passive) hemidiaphragm. However, although the relaxation length of the muscle at 20-cmH₂O transrespiratory pressure was 18.1 ± 3.4% shorter than that at FRC (P < 0.02), the fractional shortening of the contracting muscle fibers during both unilateral and bilateral stimulation was smaller. More importantly, whereas the fractional muscle shortening during unilateral stimulation at FRC tended to be greater than that during bilateral stimulation (−40.8 ± 1.0 vs. −35.2 ±
DISCUSSION

In an earlier study of the effect of inflation on the mechanics of the diaphragm, Minh et al. (13) reported that the $\Delta P_{ao}$ measured during unilateral 100-Hz stimulation of the C5-C6 phrenic nerve roots in dogs was 35–40% of the $\Delta P_{ao}$ measured during bilateral stimulation at all lung volumes. This finding argued against our hypothesis that the decrease in muscle tension associated with an increase in lung volume would induce a prominent decrease in the degree of synergism between the left and right hemidiaphragms. However, the $\Delta P_{ao}$ values obtained by these investigators were remarkably small, about one-half the values obtained in the present study and usually reported for the canine diaphragm (4, 5, 8). Such a difference suggests that Minh et al. may have caused some damage to the nerves during the surgical preparation, making it difficult to make a reliable quantitative assessment of the mechanical interaction between the two halves of the diaphragm. Yet, in agreement with the observations of Minh et al., the $\Delta P_{ao}$ measured during bilateral phrenic nerve stimulation in our animals was 50–60% greater than the predicted value at all lung volumes, including when transrespiratory pressure was set between 20 and 30 cmH₂O (Fig. 2). The measured $\Delta P_{ao}$ was also invariably greater than the predicted value (Fig. 3). It must be concluded, therefore, that, for a given muscle tension, the degree of synergism between the left and right hemidiaphragms is actually greater at high lung volumes than at FRC.

The fact that synergism decreases with decreasing muscle tension at FRC but not at higher lung volumes could be explained, at least in part, on the basis of the length-tension properties, as shown in Fig. 5. The solid lines in Fig. 5A are the active and passive length-tension curves obtained from isolated diaphragmatic muscle bundles (7, 9, 12); active force in the diaphragm, as for any skeletal muscle, decreases gradually as muscle length decreases, and it approaches zero when muscle length is ~40% of the optimal force-producing length ($L_o$). The length of the diaphragm and the force developed by the muscle during a maximal contraction in vivo, however, is also determined by the load imposed on the muscle by the lung and

Table 1. Changes in diaphragmatic muscle length during bilateral and unilateral phrenic nerve stimulation at FRC and 20-cmH₂O transrespiratory pressure

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Bilateral Stimulation</th>
<th>Unilateral Stimulation</th>
<th>$\Delta P_{ao}$, Measured/Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRC, %Le_{FRC}</td>
<td>Ipsilateral</td>
<td>Contralateral</td>
</tr>
<tr>
<td>1</td>
<td>-42.9</td>
<td>-28.6</td>
<td>+19.2</td>
</tr>
<tr>
<td>2</td>
<td>-29.4</td>
<td>-33.4</td>
<td>+18.7</td>
</tr>
<tr>
<td>3</td>
<td>-31.5</td>
<td>-28.7</td>
<td>+14.8</td>
</tr>
<tr>
<td>4</td>
<td>-37.0</td>
<td>-22.4</td>
<td>+17.3</td>
</tr>
</tbody>
</table>

These values are the individual changes in length for the right and left hemidiaphragms in 4 animals. The length changes are expressed as percentage changes relative to muscle length during relaxation at resting end expiration ($L_{e_{FRC}}$) or to muscle length during relaxation at 20-cmH₂O transrespiratory pressure ($L_{e_{20}}$). Negative changes indicate muscle shortening below relaxation length, and positive changes indicate muscle lengthening beyond relaxation length. $\Delta P_{ao}$, change in airway opening pressure; FRC, functional residual capacity.

3.0%; $P < 0.2$), at 20 cmH₂O two of four animals (dogs 3 and 4) had slightly greater fractional shortening during bilateral stimulation. For the animal group, therefore, the fractional muscle shortening during unilateral stimulation at 20 cmH₂O was nearly the same as that during bilateral stimulation ($-28.3 \pm 2.3$ vs. $-27.6 \pm 1.9\%$).

In addition, unilateral stimulation at FRC caused the contralateral hemidiaphragm to move 14.9 ± 3.1 mm in the caudal direction, but unilateral stimulation at 20-cmH₂O transrespiratory pressure caused this hemidiaphragm to move cranially in every animal (Fig. 4). For the four animals, this cranial displacement was 4.5 ± 1.3 mm. The fractional lengthening of the contralateral hemidiaphragm at 20 cmH₂O was also larger than that at FRC ($+17.5 \pm 1.0\%$ vs. $+10.6 \pm 1.8\%; P < 0.05$).

![Fig. 4. Contours of the diaphragm seen on anteroposterior radiographs in a representative animal during relaxation, during isolated tetanic stimulation of the left phrenic nerve (dashed line), and during combined stimulation of the left and right phrenic nerves at functional residual capacity (FRC; A) and after passive inflation to a transrespiratory pressure of 20 cmH₂O (B).](http://jap.physiology.org/Downloadedfrom)
the chest wall. In fact, the particular values of length and force that eventually occur when the diaphragm contracts maximally are given by the intersection of the active length-tension curve and the load curve (see Appendix), and the dashed lines in Fig. 5A represent the two load curves corresponding to maximal bilateral and unilateral diaphragmatic contraction at FRC. These load curves were established on the basis of two elements. First, because, in supine dogs, the relaxed muscle at FRC is close to $L_0$ (7, 15), the load curves must intersect the curve corresponding to the passive diaphragm at $L_0$; force at this point, expressed as a fraction of maximal force, is 0.05. Second, our recent radiographic studies (4) have shown that the contracting diaphragmatic muscle fibers at FRC shorten, on average, by 36.0% of their relaxation length during maximal bilateral stimulation of the phrenic nerves and by 41.7% of their relaxation length during maximal unilateral stimulation; similar changes in muscle length were obtained in the present study (Table 1). Consequently, at equilibrium, muscle length during bilateral contraction was taken as 0.64 $L_0$, and muscle length during unilateral contraction was taken as 0.58 $L_0$. As a result, the force developed by the muscle in such conditions would amount, respectively, to 0.35 and 0.26 of maximal force, and the ratio of the force during bilateral contraction to the force during unilateral contraction would be 1.35.

A decrease in the level of diaphragmatic activation at FRC, as is the case with a decrease in the frequency of phrenic nerve stimulation, would shift the active length-tension curve downward, such that force during isometric contraction at $L_0$ would be reduced, for example, to 0.4 of maximal force (Fig. 5B). In this condition, the force developed by the muscle during bilateral and unilateral contraction would be, respectively, 0.21 and 0.17 of maximal force, and the ratio between the two values would be only 1.23, in agreement with the observed decrease in the degree of synergism (4). On the other hand, the main effect of passive inflation would be to decrease the muscle relaxation length, for example, to 0.8 $L_0$, and, hence, to shift the load curves downward (Fig. 5C). If we assume that the load curves in this condition are parallel to those at FRC, then the force developed during maximal bilateral and unilateral contraction would be, respectively, 0.24 and 0.17 of maximal

---

Fig. 5. Graphical analysis of the force developed by the diaphragm during bilateral vs. unilateral contraction. A: maximal diaphragmatic activation at FRC. The solid lines are the force-length relationships for the maximally active diaphragm and the passive diaphragm, and the dashed lines are the load curves describing the load imposed on the diaphragm by the lung and chest wall during bilateral and unilateral contraction. Force is expressed as a fraction of maximum, and muscle length is expressed as a fraction of optimal length ($L_0$). The forces generated by the diaphragm during contraction are given by the intersection of the load curves with the active length-tension curve (●). B: predicted effect of a decrease in the level of diaphragmatic activation. With such a decrease, the force exerted by the muscle at any given length is reduced (in the example shown, force at $L_0$ is 0.4 of maximum force). The load curves, however, remain unchanged, and the forces generated by the diaphragm during bilateral and unilateral contraction are given by the new intersections (○). C: predicted effect of passive inflation. The load curves are shifted downward, such that, during maximal bilateral and unilateral contraction, length and force are reduced (○).

---

Fig. 6. Mechanical system consisting of a spring attached to a muscle. A: when the muscle contracts, it shortens and extends the spring. B: the force exerted by the muscle decreases as it shortens and the spring extends, whereas the force exerted by the spring increases.
force. In other words, the forces developed by the diaphragm during maximal activation after passive inflation would be nearly the same as those developed during submaximal activation at FRC, but the ratio of the force during bilateral contraction to that during unilateral contraction would be increased to 1.44, rather than decreased to 1.23. The ratio would be increased further if the active tension for the diaphragm in situ showed a steeper dependence on muscle length at short muscle lengths, as reported by Hubmayr et al. (8) and Boriek et al. (2).

In agreement with this idea, the radiographic measurements obtained in experiment 2 showed that the diaphragm was shorter during maximal contraction at 20-cmH₂O transrespiratory pressure than during maximal contraction at FRC (Table 1). These measurements also showed, however, that the amount of muscle shortening during unilateral contraction at 20 cmH₂O was similar to that during bilateral contraction. Although the markers were placed in the coronal midplane, rather than along muscle bundles, and although the positions of the markers were assessed only in anteroposterior projections, it, therefore, appears that the length-tension properties of the muscle are not the main determinants of the greater interhemidiaphragmatic synergism at high lung volumes. On the other hand, whereas unilateral contraction at FRC induced caudal displacement of the contralateral hemidiaphragm, unilateral contraction at 20 cmH₂O caused this hemidiaphragm to move cranially (Fig. 4). Such a cranial displacement should reduce the volume swept out and the pressure generated by the contracting hemidiaphragm. As a result, the predicted ΔPao and ΔPab should be decreased relative to the measured values.

The question, therefore, arises as to which mechanism or mechanisms induce a greater distortion of diaphragmatic configuration during unilateral phrenic nerve stimulation at high lung volumes. In a recent study of diaphragm motion in dogs with acute hemidiaphragmatic paralysis, Scillia et al. (16) concluded that the motion of the inactive hemidiaphragm at FRC is largely determined by the balance between the force generated by the intact hemidiaphragm and the force related to the fall in pleural pressure (ΔPpl). Thus, when the latter force outweighs the former, as is the case during occluded breaths involving the simultaneous contraction of one hemidiaphragm and all the inspiratory intercostal muscles, the inactive hemidiaphragm moves cranially. Conversely, when the force generated by the intact hemidiaphragm is predominant, such as during isolated stimulation of one phrenic nerve, then the inactive hemidiaphragm moves caudally. To be sure, during unilateral phrenic nerve stimulation at high lung volumes, the contralateral hemidiaphragm would continue to move caudally in much the same way as it does when the frequency of stimulation is reduced at FRC (16).

There is, however, a large difference between the passive tension generated by the inactive hemidiaphragm at FRC and that generated at 20-cmH₂O transrespiratory pressure. During unilateral phrenic nerve stimulation at FRC, the inactive hemidiaphragm lengthened, on average, by 10% (Table 1). Muscle length during stimulation, therefore, was ~1.10 Lₒ. In contrast, when transrespiratory pressure was set at 20 cmH₂O, the relaxed muscle was shortened to 82% of its FRC length. During unilateral phrenic nerve stimulation, therefore, even though the fractional lengthening of the inactive hemidiaphragm was increased to 17.5%, muscle length was only 0.96 Lₒ. Consequently, the amount of passive tension in this hemidiaphragm was much smaller than at FRC, as shown in Fig. 5A. Specifically, the passive length-tension curves previously obtained from canine diaphragmatic strips in situ indicate that passive tension at 0.96 Lₒ is ~40% of that at 1.10 Lₒ (9). Measurements of pressure across the canine diaphragm during abdominal compression (15) or after the injection of large amounts of liquid in the abdominal cavity (8) similarly indicate that passive tension at 0.96 Lₒ is only 20–40% of passive tension at 1.10 Lₒ. In this condition, for a similar tension in the contracting hemidiaphragm and a similar ΔPpl, the displacement of the inactive hemidiaphragm would be less caudal or more cranial.

APPENDIX

Figure 6A shows a simple mechanical system consisting of a muscle attached to a spring. When the muscle in the system is activated, it shortens and extends the spring upward. The force exerted by the muscle decreases as it shortens, but at the same time the (downward) force exerted by the spring increases as it lengthens (Fig. 6B). At equilibrium, the changes in length of the spring and the muscle are equal and the forces developed by them are also equal. The solution, therefore, is given by the intersection of the two length-force curves. The load curves for the diaphragm during bilateral and unilateral contraction should be analogous to the load curve for the muscle in Fig. 6, and they should also relate force to length, as shown in Fig. 5A.

ACKNOWLEDGMENTS

The authors are grateful to T. A. Wilson for helpful discussions.

GRANTS

The authors are grateful to the Fonds National de la Recherche Scientifique (Belgium) for its support (Grant 3.4509.04).

REFERENCES


