Effect of diaphragmatic contraction on the action of the canine parasternal intercostals

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De Troyer, André and Dimitri Leduc. Effect of diaphragmatic contraction on the action of the canine parasternal intercostals. J Appl Physiol 101: 169–175, 2006; doi:10.1152/japplphysiol.01465.2005.—The inspiratory intercostal muscles enhance the force generated by the diaphragm during lung expansion. However, whether the diaphragm also alters the force developed by the inspiratory intercostals is unknown. Two experiments were performed in dogs to answer the question. In the first experiment, external, cranially oriented forces were applied to the different rib pairs to assess the effect of diaphragmatic contraction on the coupling between the ribs and the lung. The fall in airway opening pressure (ΔPao) produced by a given force on the ribs was invariably greater during phrenic nerve stimulation than when the diaphragm relaxed. The cranial rib displacement (Xr), however, was 40–50% smaller, thus indicating that the increase in ΔPao was exclusively the result of the increase in diaphragmatic elastance. In the second experiment, the parasternal intercostal muscle in the fourth interspace was selectively activated, and the effects of diaphragmatic contraction on the ΔPao and Xr caused by parasternal activation were compared with those observed during the application of external loads on the ribs. Stimulating the phrenic nerves increased the ΔPao and reduced the Xr produced by the parasternal intercostals, and the magnitudes of the changes were identical to those observed during external rib loading. It is concluded, therefore, that the diaphragm has no significant synergistic or antagonistic effect on the force developed by the parasternal intercostals during breathing. This lack of effect is probably related to the constraint imposed on intercostal muscle length by the ribs and sternum.

respiratory muscles; muscle interaction; chest wall mechanics; rib-lung coupling

IT IS NOW WELL ESTABLISHED, both in humans (4, 9, 10, 20, 30) and in animals (11, 21, 25, 26, 29), that the intercartilaginous portion of the internal intercostal muscles (the so-called parasternal intercostals) and the external intercostal muscles contract in concert with the diaphragm during the inspiratory phase of the breathing cycle (see Ref. 12 for review). In fact, these intercostal muscles contribute to the expansion of the lung by elevating the ribs and expanding the rib cage. In addition, by causing pleural pressure (Ppl) to fall, they also prevent the diaphragmatic muscle fibers from shortening excessively (6, 17). Because the pressure-generating ability of these fibers depends closely on their length during contraction (22, 23, 28), the diaphragm therefore develops greater pressure during simultaneous diaphragm-intercostal contraction than it does during isolated contraction. And indeed, in the dog, the inspiratory intercostal muscles and the diaphragm do act synergistically on the lung during breathing (6, 17). However, whether the diaphragm also enhances the force developed by the inspiratory intercostals during inspiration is unknown.

Previous studies of the mechanical interactions among the intercostal muscles have suggested that the insertions of the muscles on the ribs, the costal cartilages, and the sternum impose significant constraint on their length and, with it, on their force-generating ability. Specifically, in the dog with the airway occluded at 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 (27). 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 contraction (2). On the other hand, when the phrenic nerves in dogs (8) and in humans (1) are selectively stimulated, the ΔPao obtained during bilateral stimulation is clearly greater than the sum of the ΔPao values obtained during separate left and right stimulation. Thus, apparently, in contrast to the diaphragm, the intercostal muscles have small enough changes in length and remain near enough to their optimum force-producing lengths that the synergistic or antagonistic interactions between them are negligible. On this basis, therefore, it would be reasonable to speculate that the force-generating ability of the intercostal muscles remains essentially unchanged during contraction of the diaphragm.

In the present studies, the hypothesis was thus tested that the diaphragm causes little or no alteration in the force-generating ability of the canine parasternal intercostals. The parasternal intercostals were selected for the study because they are more easily accessible than the external intercostals and also because, in the dog, they are the main determinant of the inspiratory elevation of the ribs (5). The force generated by the parasternal intercostals during breathing, however, cannot be measured directly. Furthermore, because an isolated contraction of these muscles causes a (passive) cranial displacement of the diaphragm, which corresponds to a pressure dissipation, it would be expected that a particular force generated by the parasternal intercostals in the presence of diaphragmatic activity would lead to a greater fall in Ppl than if the same parasternal force were generated with the diaphragm relaxed. If so, it would also be difficult to estimate the force developed by these muscles on the basis of Ppl measurements alone. To
C5 and C6 phrenic nerve roots were isolated bilaterally in the neck and (Pab). After the abdominal wall was tightly sealed in two layers, the between the liver and the stomach to measure abdominal pressure a midline incision from the xiphisternum to the umbilicus, and a sides of the chest from the 1st to the 11th rib by reflection of the skin after which the rib cage and intercostal muscles were exposed on both sides of the chest from the 1st to the 11th rib by reflection of the skin and the superficial muscle layers. The abdomen was then opened by a midline incision from the xiphisternum to the umbilicus, and a balloon-catheter system filled with 1.0 ml of air was positioned between the liver and the stomach to measure abdominal pressure (Pab). After the abdominal wall was tightly sealed in two layers, the C5 and C6 phrenic nerve roots were isolated bilaterally in the neck and laid over two pairs of insulated stainless steel stimulating electrodes, and a differential pressure transducer (Validyne, Northridge, CA) was connected to a side port of the endotracheal tube to measure Pao.

Three experimental protocols were then followed. Experiment 1. Six animals were studied first to assess the effect of diaphragmatic contraction on the coupling between the ribs and the lung. The method used to determine this coupling has been previously described (13, 16). Thus hooks were screwed into the fourth right and left bony ribs, 1 cm lateral to the costochondral junctions. A long inextensible thread was attached to each hook and led cranially, parallel to the longitudinal body axis of the animal, over a pulley placed at the head of the table, and it was connected to a small basket in which weights could be placed later. An additional hook was screwed into the fourth right rib in the midaxillary line and connected to a linear displacement transducer (Schaevitz Engineering, Pennsauken, NJ) to measure the craniocaudal (axial) rib displacement (11). After the animal was made apneic by mechanical hyperventilation, the endotracheal tube was occluded at resting end expiration (FRC), and 200-g lead balls were placed in both baskets attached to the fourth rib so that the load in each basket was increased by 0.2-kg increments from 0.2 to 0.8 kg. Two runs of loading were performed. The animal was then reconnected to the ventilator and hyperventilated, and square pulses of 0.1-ms duration and supramaximal voltage were applied at a frequency of 20 impulses/s to the left and right C5, and C6 phrenic nerve roots. The stimulation was maintained for 4–8 s, at which time the endotracheal tube was occluded and loads were applied again to the fourth rib pair. This time interval allowed the respiratory system to reach equilibrium (i.e., the diaphragm after 4–8 s caused no further change in Pao), such that the effects of the loads on the rib and Pao could be accurately determined. To reduce the total number of phrenic nerve stimulations and maintain diaphragmatic contractility constant throughout the experiment, only two loads, namely 0.4 and 0.8 kg, were applied in this condition. As during control, however, two trials were obtained with each load.

After completion of these measurements, the two hooks and baskets and the displacement transducer were transferred to the fifth rib pair, and two runs of loading were performed, first with the diaphragm relaxed and then during tetanic stimulation of the C5–C6 phrenic nerves. The procedure was subsequently repeated for each individual rib pair down to the 10th pair. For the fifth rib pair, however, an additional thread was attached to the hook in the midaxillary line and led laterally, perpendicular to the sagittal midplane, to measure both the lateral and the axial rib displacement (11).

Experiment 2. Six animals were studied next to assess the effect of diaphragmatic contraction on the force generated by the parasternal intercostals. Two successive procedures were used in each animal. The first procedure was similar to that used in experiment 1 and involved applying cranially oriented loads to the fifth rib pair, first with the diaphragm relaxed and then during tetanic stimulation of the phrenic nerves. Next the parasternal intercostal muscle in the fourth interspace was selectively activated by using a pair of stimulating electrodes implanted in the muscle on either side of the sternum. These electrodes were silver hooks insulated with polyethylene tubing except for their terminal 8 mm, and their implantation was made superficially along muscle bundles situated in the vicinity of the sternum, i.e., in the area of the parasternal intercostal muscle with the greatest respiratory mechanical advantage (14). The internal intercostal nerve in the fourth interspace was also sectioned at the chondrocostal junction so as to avoid both antidromic stimulation of the internal intercostal muscle in the fourth interspace and stimulation, through spindle afferents, of intercostal muscles in contiguous segments (18, 24).

The animal was allowed to recover for 15–20 min after instrumentation, after which it was made apneic by mechanical hyperventilation. Square pulses of 0.2-ms duration and 20-Hz frequency were then delivered at intervals to the parasternal intercostal muscle. The stimulus intensity was increased by increasing the voltage of stimulation. At least four voltages, usually between 6 and 15 V, were used in each animal, and for each voltage, two trials of stimulation with the diaphragm relaxed alternating with two trials of stimulation superimposed on tetanic phrenic nerve stimulation were performed. The sequence of events in such trials was the same as that used for external rib loading. That is, with the animal apneic, stimulation was delivered to the phrenic nerves, and when equilibrium was achieved, the endotracheal tube was occluded and the parasternal intercostal was stimulated.

Experiment 3. Stimulating the phrenic nerves induced a marked caudal displacement of the ribs (see RESULTS). Consequently, the parasternal intercostal muscles before contraction were longer during phrenic nerve stimulation than with the diaphragm relaxed, and this difference in length could alter the force-generating ability of the muscles independent of any effect of diaphragmatic contraction. Six animals were studied to overcome the potential influence of this confounding factor. The procedure initially was identical to that described in experiment 2 and involved cranial loading of the fifth rib pair with the diaphragm relaxed and during tetanic stimulation of the C5–C6 phrenic nerve roots. The parasternal intercostal muscle in the fourth interspace was also activated with the diaphragm relaxed. At this stage, however, a nonelastic strap was placed around the abdomen from the pubis to the rib cage margin to increase abdominal elastance and to reduce the caudal rib displacement during phrenic nerve stimulation. External loading of the fifth rib pair and activation of the parasternal intercostal during phrenic stimulation were then repeated.

The animals were maintained at a constant, rather deep level of anesthesia throughout the study. They had no corneal reflex, no limb movement, and no changes in blood pressure and heart rate, including during phrenic nerve stimulation. At the conclusion of the measurements, the animal was given an overdose of anesthetic (30–40 mg/kg iv).

Data analysis. For each rib in each animal of experiment 1, the changes in Pao (ΔPao) and the axial rib displacements (Xr) induced by each load [force (F)] with the diaphragm relaxed and during phrenic nerve stimulation were averaged over the two runs. The relationships thus obtained between ΔPao and F and between Xr and F were then calculated by using linear regression techniques (coefficient of corre-
RESULTS

The slopes of the relationships between $\Delta P_{ao}$ and $F$ and between $X_r$ and $F$ obtained for all the ribs during loading with the diaphragm relaxed (control) and during loading in the presence of phrenic nerve stimulation are shown in Fig. 1. In agreement with our laboratory’s previous observations (13, 16), $\Delta P_{ao}/F$ during control increased from the 4th to the 6th rib pair and then decreased from the 6th to the 10th rib pair (Fig. 1A), whereas $X_r/F$ increased continuously ($P < 0.001$) from the 5th to the 10th rib (Fig. 1B). With phrenic nerve stimulation, Pab in the six animals increased by $6.8 \pm 0.4$ cmH$_2$O and the ribs moved caudally relative to their resting, end-expiratory position; the magnitude of this displacement varied with rib number and ranged between $5.27 \pm 0.50$ mm (rib 4) and $8.00 \pm 0.72$ mm (rib 9). When the ribs were loaded in this condition, however, $\Delta P_{ao}/F$ was consistently greater than during control ($P < 0.001$), whereas $X_r/F$ was consistently smaller ($P < 0.001$). As shown in Fig. 2, the loss in $X_r/F$, when expressed as a percentage of the control value, was relatively uniform across the rib cage. In contrast, the increase in $\Delta P_{ao}/F$ was similar for ribs 4–6 but then increased markedly from rib 7 to rib 10 ($P < 0.001$).

The pattern of rib motion observed during loading of the fifth rib pair in the control condition and in the presence of phrenic nerve stimulation is shown in Fig. 3. Loading in both conditions caused the ribs to move cranially and outward. Although both the cranial and the outward rib displacements induced by a given load were smaller in the presence of phrenic nerve stimulation than during control, the outward displacement associated with a given cranial displacement was similar.

The records of $\Delta P_{ao}$ and $X_r$ obtained in a representative animal during bilateral contraction of the fourth parasternal intercostal muscle in the control condition and during the same parasternal intercostal contraction superimposed on phrenic nerve stimulation are shown in Fig. 4A, and the values of $\Delta P_{ao}$ and $X_r$ obtained for all stimulation intensities in the same animal are shown in Fig. 4B and C, respectively. Stimulating the parasternal intercostal in the control condition (●) produced a cranial displacement of the fifth rib and a fall in Pao, and these effects increased in magnitude as the intensity of stimulation was increased. A similar pattern was observed when stimulation of the parasternal intercostal was superimposed on phrenic nerve stimulation (○). For any given stimulation intensity, however, $\Delta P_{ao}$ was greater when parasternal contraction was superimposed on phrenic nerve stimulation.
than it was during control. In contrast, Xr was consistently smaller. In agreement with experiment 1, however, when external forces were applied to the fifth rib pair in this particular animal, ΔPao/F increased from −1.80 cm H2O/kg during control to −2.70 cm H2O/kg during phrenic nerve stimulation, and Xr/F decreased from +8.70 to +6.00 mm/kg. If the force generated by the parasternal intercostal muscle were unaltered by phrenic nerve stimulation, one would therefore predict that the ΔPao produced by this muscle would increase by 50% with phrenic stimulation, whereas Xr would decrease by 46%. The predicted values of ΔPao and Xr are represented by the plus signs in Fig. 4, B and C. As can be seen, the measured values agreed well with the predicted values. When the data obtained for all stimulation intensities were averaged, the measured and predicted values of ΔPao in this animal thus amounted to −2.14 and −2.23 cm H2O, respectively; the measured and predicted values for Xr were +1.30 and +1.17 mm.

Similar results were obtained in all animals of experiment 2, as shown in Fig. 5. Thus, for the six animals, ΔPao during parasternal contraction in the control condition was −2.45 ± 0.28 cm H2O. and Xr was +4.63 ± 0.76 mm. When the phrenic nerves were stimulated, causing a rise in Pab of +9.2 ± 0.5 cm H2O and a caudal rib displacement of 5.83 ± 0.39 mm, ΔPao during parasternal contraction was increased to −3.22 ± 0.29 cm H2O (P < 0.001), and Xr was reduced to +2.40 ± 0.31 mm (P < 0.01). These two values, however, were not different from the predicted values (ΔPao: −3.17 ± 0.26 cm H2O; Xr = +2.63 ± 0.37 mm).

With abdominal strapping (experiment 3), the ΔPab induced by phrenic stimulation in the six animals increased from +8.6 ± 0.7 to +25.3 ± 3.0 cm H2O (P < 0.01), and the caudal displacement of the fifth rib decreased from 5.07 ± 0.65 to 0.58 ± 0.36 mm (P < 0.001). Strapping the abdomen, however, did not affect ΔPao/F or ΔPab/F during rib loading in the presence of phrenic stimulation. In addition, the effects of phrenic stimulation on the alterations induced by contraction of the parasternal intercostal were similar to those observed in experiment 2 (i.e., without abdominal strapping). Thus the ΔPao produced by contraction of the fourth parasternal intercostal increased from −2.44 ± 0.25 cm H2O during control to −3.44 ± 0.31 cm H2O during phrenic stimulation (P < 0.01), Xr was reduced from +4.31 ± 0.52 to +2.33 ± 0.31 mm (P < 0.01), and the measured values of ΔPao and Xr, here too, were nearly the same as the predicted values (Fig. 5).

DISCUSSION

The first important result of this study is the demonstration that cranially oriented forces on the ribs produce greater falls in Pao when the phrenic nerves are stimulated than when the diaphragm is relaxed (Fig. 1A). For a given force, however, Xr was reduced by 40–50% (Figs. 1B and 2). Presumably, the increase in ΔPao during phrenic stimulation would have been larger had the cranial rib displacement been maintained. Furthermore, previous studies have shown that in the dog, rib displacement in the outward direction is about four times more effective in causing lung expansion than rib displacement in the cranial direction (13, 15). Consequently, an increase in the ratio of the outward rib displacement over the Xr during phrenic stimulation could have accounted for the increase in ΔPao. Rib loading during phrenic stimulation, however, drove the ribs along the same trajectory as it did with the diaphragm relaxed (Fig. 3). The increase in ΔPao must, therefore, be primarily related to the increase in diaphragmatic elastance.

Fig. 2. Mean ± SE values of ΔPao/F (A) and Xr/F (B), expressed as percentages of the control values, obtained during cranial loading of the individual rib pairs in the presence of phrenic nerve stimulation. Note the progressive increase in ΔPao/F from rib 7 to rib 10.
Although the increase in $\Delta P_{\text{ao}}$ during phrenic stimulation was seen for all the ribs, it is noteworthy that this increase was particularly prominent for the most caudal ribs (Fig. 2A). This result, in fact, supports our laboratory’s previous suggestion that the effect of a particular rib on the lung is directly related to the area of the lung subtended by the rib (16). Thus, at FRC, whereas the ribs in the cranial half of the rib cage are apposed to the lung, the most caudal ribs are apposed to the abdomen through the diaphragm. Therefore, with the diaphragm relaxed, a cranially oriented force on these ribs results primarily in an expansion of the abdominal wall and a fall in $P_{\text{ab}}$, and the fall in $P_{\text{ao}}$ is small, due to the small passive caudal displacement of the diaphragm. On the other hand, when the phrenic nerves were stimulated, there was a marked caudal displacement of the diaphragm, as shown by the large rise in $P_{\text{ab}}$. Previous radiographic measurements of diaphragmatic configuration in dogs have shown that with such phrenic nerve stimulation, the zone of apposition of the diaphragm to the rib cage is actually abolished (7, 8). In this condition, therefore, the most caudal ribs are also largely apposed to the lung, and external forces applied to them in the cranial direction can also generate significant falls in $P_{\text{ao}}$. 

![Figure 4](image-url) Fig. 4. Effect of diaphragmatic contraction on the inspiratory action of the parasternal intercostal muscles. A: records of $\Delta P_{\text{ao}}$ and $X_r$ obtained in a representative animal during bilateral stimulation (15 V) of the parasternal intercostal muscle in the 4th interspace with the diaphragm relaxed (left) and during the same parasternal intercostal stimulation superimposed on tetanic stimulation of the phrenic nerves (right). B and C: values of $\Delta P_{\text{ao}}$ and $X_r$ obtained for all stimulation intensities in the same animal. ○, Data obtained with the diaphragm relaxed (control); ◯, data obtained during phrenic stimulation; +, data that would be obtained if phrenic stimulation did not alter the force generated by the parasternal intercostal muscle (predicted). Note that for each stimulus intensity, $\Delta P_{\text{ao}}$ is greater during phrenic stimulation than during control (B), whereas $X_r$ is smaller (C). However, the values measured during phrenic nerve stimulation agree well with the predicted values.

![Figure 5](image-url) Fig. 5. Comparison between the predicted and measured values of $\Delta P_{\text{ao}}$ (A) and between the predicted and measured values of $X_r$ (B) obtained in 12 animals during contraction of the parasternal intercostal muscle in the 4th interspace superimposed on phrenic nerve stimulation. Stimulation of the phrenic nerves was performed with no restraint on abdominal motion in 6 animals (●) and with a nonelastic strap placed around the abdomen in the other 6 animals (○). Solid line, line of identity. Note that the measured values for both $\Delta P_{\text{ao}}$ and $X_r$ agree well with the predicted values in all animals.
On the basis of these findings, it was expected that unless the force generated by the parasternal intercostals was markedly reduced by diaphragmatic contraction, the $\Delta P_{ao}$ produced by a given parasternal activation would be greater during phrenic nerve stimulation than with the diaphragm relaxed. In agreement with this prediction, all animals in experiment 2 showed indeed a greater $\Delta P_{ao}$ when contraction of the parasternal intercostals was superimposed on phrenic nerve stimulation. The second important result of this study, however, is the observation that this increase in $\Delta P_{ao}$ was similar in magnitude to that observed when external loads were applied to the ribs (Figs. 4 and 5). The adverse effect of phrenic nerve stimulation on the Xr produced by the parasternal intercostal was also quantitatively similar to that observed during the application of external loads, thus indicating that in this experimental condition, the beneficial effect of diaphragmatic contraction on the lung expanding action of the parasternal intercostals was entirely related to the increase in diaphragmatic elastance. In other words, the force generated by the parasternal intercostals appeared to be unaffected by contraction of the diaphragm.

A possible complication to this experiment is that contraction of the parasternal intercostals was initiated well after the diaphragm was fully activated. As previously pointed out for the diaphragm (see the introduction), the force developed by a particular muscle during contraction is primarily determined by its length, and muscle length, in turn, is determined by the length-tension characteristics of the muscle and by the load imposed on the muscle (7). Therefore, there is no reason to believe that the late contraction of the parasternal intercostal would, by itself, alter the force generated by the muscle. However, the early, isolated stimulation of the phrenic nerves caused a marked caudal displacement of the ribs, thus implying that the parasternal intercostals before contraction were lengthened and displaced away from their in vitro optimum force-producing length (19). In addition, the rib cage in the dog becomes less compliant when it contracts below its resting, end-expiratory volume (3, 8). Therefore, the rib cage during phrenic nerve stimulation was also stiffer than it was with the diaphragm relaxed, so it would be expected that a given force on the ribs would induce a smaller rib displacement. And indeed, during phrenic stimulation in experiment 2, the Xr produced both by external forces on the ribs and by activation of the parasternal intercostals was invariably decreased (Figs. 1B and 4C). Consequently, the shortening of the muscles during contraction was reduced, and this reduction, combined with the increase in muscle length before contraction, might have obscured any synergistic or antagonistic effect that the diaphragm may have otherwise on these muscles.

When the abdomen was bound with nonelastic straps and the ribs during phrenic stimulation were maintained near their FRC position (experiment 3), however, the effects of diaphragmatic contraction on $\Delta P_{ao}$ and Xr produced by the parasternal intercostals remained quantitatively similar to the effects of diaphragmatic contraction on the changes produced by external forces on the ribs (Fig. 5). The abdominal straps, however, did not alter the rib or the pressure response to such external forces, thus suggesting that they did not impact on the action of the parasternal intercostal muscle being studied. In agreement with our hypothesis, therefore, the conclusion can be drawn that diaphragmatic contraction does not significantly alter the force generated by the parasternal intercostals. Furthermore, to the extent that this lack of effect would be the result of the constraint imposed on the parasternal intercostals by the sternum and the costal cartilages, it would be expected that this conclusion would also apply to humans regardless of the difference in shape between the canine and the human rib cage.

The question remains, however, as to why the Xr associated with a particular activation of the parasternal intercostals was still reduced when phrenic stimulation was performed with the abdomen bound (experiment 5). Because the ribs in this condition were maintained near their FRC position, it was expected that rib cage elastance would be unaltered and, hence, that the Xr during parasternal contraction would be maintained. The mechanism for the persistent reduction in cranial rib displacement during phrenic stimulation with the abdomen bound cannot be defined at this stage, but it must be pointed out that the Xr and the fall in Pao (and in Ppl) are mutually related. To be sure, the cranial displacement of the ribs during contraction of the parasternal intercostals causes the fall in Ppl, but at the same time, the fall in pressure does oppose the Xr. Because the fall in Ppl generated by the parasternal intercostal was greater when the muscle contraction was superimposed on phrenic stimulation, the force opposing the cranial rib displacement should therefore be greater. Until the effectiveness of Ppl in opposing the displacement of the ribs is quantified, however, the role of this mechanism remains speculative.

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