The action of the canine diaphragm on the lower ribs depends on activation

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De Troyer A. The action of the canine diaphragm on the lower ribs depends on activation. J Appl Physiol 111: 1266–1271, 2011. First published July 14, 2011; doi:10.1152/japplphysiol.00402.2011.—Conventional wisdom maintains that the diaphragm lifts the lower ribs during isolated contraction. Recent studies in dogs have shown, however, that supramaximal, tetanic stimulation of the phrenic nerves displaces the lower ribs caudally and inward. In the present study, the hypothesis was tested that the action of the canine diaphragm on these ribs depends on the magnitude of muscle activation. Two experiments were performed. In the first, the C5 and C6 phrenic nerve roots were selectively stimulated in 6 animals with the airway occluded, and the level of diaphragm activation was altered by adjusting the stimulation frequency. In the second experiment, all the inspiratory intercostal muscles were severed in 7 spontaneously breathing animals, so that the diaphragm was the only muscle active during inspiration, and neural drive was increased by a succession of occluded breaths. The changes in airway opening pressure and the craniocaudal displacements of ribs 5 and 10 were measured in each animal. The data showed that 1) contraction of the diaphragm causes the upper ribs to move caudally; 2) during phrenic nerve stimulation, the lower ribs move cranially when the level of diaphragm activation is low, but, if the lower rib cage expansion observed during selective activation of the costal portion of the diaphragm in dogs (12). Selective activation of the crural part of the canine diaphragm, however, produced no displacement of the lower rib cage (12), and these observations, combined with theoretical considerations (23), have led to the conventional view that the diaphragm has an inspiratory action on the lower rib cage and that this action is the result of two mechanisms. First, because the muscle fibers of the costal diaphragm insert on the lower ribs and run cranially from these insertions, they exert a cranially oriented force on these ribs when they contract. This force, conventionally referred to as the “insertional” force, has therefore the effect of lifting the lower ribs and rotating them outward (12, 23). Second, inasmuch as the muscle fibers of the costal diaphragm run cranially from their insertions on the ribs, they are also directly apposed to the inner aspect of the lower rib cage and constitute the so-called “zone of apposition” (24). This arrangement allows the rise in Pab to be transmitted across the diaphragm to the lower rib cage.

Contrary to this conventional view, however, studies have shown that, when the diaphragm in dogs is selectively activated by supramaximal, tetanic (20–100 Hz) stimulation of the phrenic nerves, the lower ribs move caudally (8, 9) and the cross-sectional area of the lower rib cage decreases (3). The idea could be offered that this expiratory displacement of the lower ribs is related to a species difference, but the lower rib cage expansion observed during selective activation of the costal portion of the canine diaphragm (12) argues against it. The hypothesis was raised, therefore, that this “paradoxical”, caudal displacement of the lower ribs was related to the fact that supramaximal, tetanic stimulation of the phrenic nerves produces marked shortening of the diaphragm muscle fibers and a large decrease in the zone of apposition (9). In this condition, most of the lower rib cage would be exposed to the expiratory effect of Ppl, rather than the inspiratory effect of Pab. If the zone of apposition disappeared, the normal inspiratory effect of the insertional force would also be abolished and perhaps reversed into an expiratory effect.

The objective of the present study was to test the hypothesis that the direction of displacement of the lower ribs during isolated diaphragm contraction is dependent on the level of activation of the muscle. Specifically, when activation is low, as is the case during resting breathing or phrenic nerve pacing in quadriplegic subjects, the diaphragm would displace the lower ribs cranially; on the other hand, during forceful contraction, it would displace the lower ribs caudally by exposing them to the large ΔPpl. Two sets of experiments were performed in dogs to test this hypothesis. In the first set, the phrenic nerves were selectively stimulated, and the level of muscle activation was altered by adjusting the stimulation frequency. In the second set, the animals were breathing spontaneously, and all the inspiratory intercostal muscles were

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METHODS

The studies were carried out on 13 adult bred-for-research dogs (20–36 kg) anesthetized with pentobarbital sodium (initial dose, 30 mg/kg iv), as approved by the Animal Ethics and Welfare Committee of the Brussels School of Medicine. The animals were placed in the supine position and intubated with a cuffed endotracheal tube, and a venous cannula was inserted in the forelimb to give maintenance doses of anesthetic (3–5 mg·kg⁻¹·h⁻¹ iv). The abdomen was opened by a 4-cm-long midline incision between the xiphoid process and the umbilicus, and a balloon-catheter system filled with 1.0 ml of air was placed between the stomach and the liver to measure Pab. After the abdomen was closely sutured, the rib cage and intercostal muscles were exposed on both sides of the chest from the first to eleventh rib by reflection of the skin and superficial muscle layers, and two hooks were screwed into the right fifth and tenth ribs in the midaxillary line and connected to linear displacement transducers (Schaevitz Engineering, Pennington, NJ) to measure the cranio-caudal (axial) displacement of the upper and lower ribs, respectively. This technique has been previously described (6). In each animal, a differential pressure transducer (Validyne, Northridge, CA) was also connected to a side port of the endotracheal tube to measure airway opening pressure (Pao). Two experimental protocols were then followed.

Experiment 1. Six animals were studied first to evaluate the influence of the level of activation of the diaphragm on rib motion during isolated phrenic nerve stimulation. Thus, in each animal, the C5 and C6 phrenic nerve roots were isolated bilaterally in the neck and laid over two pairs of insulated stainless steel electrodes that were connected to a stimulator (model S44, Grass, Quincy, MA). The animal was then connected to a mechanical ventilator (Harvard Pump, Chicago, IL) and made apneic by mechanical hyperventilation. After the animal was disconnected from the ventilator, the endotracheal tube was occluded at resting end-expiration (functional residual capacity, FRC), and rectangular trains of square pulses of 0.1 ms duration and supramaximal voltage were applied to the phrenic nerve roots in random order at frequencies of 10, 11, 12, 15, 18, and 20 impulses/s. Each stimulation was maintained for 2–3 s and performed in duplicate.

The animal was subsequently reconnected to the ventilator, and the C5 and C6 phrenic nerve roots were connected to a stimulator (model MP 16, Physitech Elect, Marseille, France) that delivers ramp, rather than rectangular, stimulations. Thus, although the pulses were still 0.1 ms in duration, their frequency increased gradually from 10 to 25 Hz over the 2.0-s course of the stimulation. These ramp stimulations were also performed in duplicate while the endotracheal tube was occluded at FRC.

To assess the mechanism of the caudal displacement of the lower ribs produced by isolated diaphragm contraction at high stimulation frequencies, the C5 and C6 phrenic nerve roots were finally reconnected to the Grass S44 stimulator, and an additional set of stimulation was performed with a stimulation frequency of 20 Hz. The intercostal muscles in the third interspace were then severed over ~2 cm at the chondrocostal junction to induce bilateral pneumothoraces, and a final set of 20-Hz stimulation of the phrenic nerves was performed.

Experiment 2. Seven animals were studied next to assess the effect of spontaneous, isolated contraction of the diaphragm on the ribs. In each animal, the internal intercostal nerves in all interspaces from the first to the eighth were exposed bilaterally at the chondrocostal junctions, and all but the one in the second left interspace were sectioned. The external intercostal muscles in interspaces 1–8 were also bilaterally severed from the chondrocostal junctions to the spine. As a result, all the inspiratory intercostal muscles were eliminated from the act of breathing except for the second left parasternal intercostal. A pair of silver hook electrodes spaced 3–4 mm apart was implanted in this muscle to measure inspiratory EMG activity and, thus, to provide a time reference for rib displacement and pressure. Implantation of these electrodes was made in parallel fibers in the muscle area known to receive the greatest neural inspiratory drive, i.e., in the vicinity of the sternum (10, 22). The EMG signal was processed with an amplifier (model 830/1, CWE, Ardmore, PA), band-pass filtered below 100 and above 2,000 Hz, and rectified before its passage through a leaky integrator with a time constant of 0.2 s.

The animal was allowed to recover for 30 min after surgery, after which baseline measurements of rib displacement, ΔPao, ΔPab, and parasternal EMG activity were made. The animal was spontaneously breathing throughout. Every 10 to 15 breaths, however, the endotracheal tube was occluded at end-expiration for a single inspiratory effort. Four to five occluded breaths were recorded in each animal. After completion of these measurements, the endotracheal tube was again occluded at end-expiration, and occlusion was maintained for 15–20 successive breaths so as to increase the magnitude of the neural inspiratory drive.

Deactivation of the parasternal intercostals in all interspaces on the right side and in interspaces 3–8 and 1 on the left side was demonstrated by the suppression of inspiratory contraction of the external intercostal in the 9th interspace. In the dog and in the cat, this muscle is not used during breathing under normal circumstances (5, 15, 18, 21), but by contracting in our animals, it would exert a cranial force on rib 10 and, thus, confound the measurement. To evaluate this potential confounding factor, the electromyogram of the 9th external intercostal muscle on the right side was examined in each animal. No animal showed any inspiratory EMG activity in the muscle, including during successive occluded breaths. We can ensure, therefore, that in each animal, the diaphragm and the parasternal intercostal in the second left interspace were the only muscles active during inspiration.

The animals in experiment 1 were maintained at a constant, rather deep level of anesthesia throughout the study. They did not react to painful stimuli and made no movements of the fore- or hindlimbs, including during phrenic nerve stimulation. The animals in experiment 2 were also maintained at a rather deep level of anesthesia. Thus they had no pupillary light reflex and made no spontaneous movements other than respiratory movements both during the surgery and during the measurements, but the corneal reflex was maintained. Rectal temperature was also kept constant between 36 and 38°C with infrared lamps. At the end of the experiment, the animals were given an overdose of anesthetic (30–40 mg/kg iv).

Data analysis. For each animal of experiment 1, the values of ΔPao, ΔPab, and axial displacement of ribs 5 and 10 obtained for each stimulation frequency (rectangular trains) of the phrenic nerves were averaged over the two trials; rib displacements in the caudal direction were given a negative sign, and displacements in the cranial direction were given a positive sign. The displacements of rib 10 obtained for the different stimulation frequencies were then plotted against the corresponding ΔPao values, the relationship between these values was fitted by a linear regression equation (r value between 0.991 and 0.999), and rib displacements at fixed ΔPao values of −10, −20, −30, and −40 cmH2O were determined from this equation by interpolation. The data for rib 5 displacement were analyzed similarly, although the relationship between rib displacement and ΔPao was fitted by a quadratic, rather than linear, regression equation. The values thus obtained for rib displacements were finally averaged across the animal group, and they are presented as means ± SE.

For each single occluded breath recorded in each animal of experiment 2, the axial displacement of rib 5 and rib 10 was measured at 2-cmH2O increments of ΔPao until peak pressure was reached. These
values were then averaged over the four to five trials, and they were plotted against \( \Delta P_{\text{ao}} \). The last three inspiratory efforts recorded during the succession of occluded breaths were analyzed similarly. It is worth pointing out that all these values were measured relative to the onset of the inspiratory burst in the parasternal intercostal of the second left interspace. Consequently, the rib displacements and \( \Delta P_{\text{ao}} \) that were considered in the data analysis resulted exclusively from the contraction of the diaphragm (and the single parasternal intercostal in the second left interspace) and were not corrupted by the relaxation of the abdominal muscles and the internal interosseous intercostals at the end of expiration.

RESULTS

Rib displacement during phrenic stimulation (experiment 1). As anticipated, the fall in \( P_{\text{ao}} \) and the rise in \( P_{\text{ab}} \) increased progressively as the frequency of stimulation of the phrenic nerves was increased from 10 to 20 Hz. Stimulation also produced a caudal displacement of rib 5, which also increased in magnitude with increasing stimulation frequency. As shown in Fig. 1, the relationship between rib displacement and \( \Delta P_{\text{ao}} \) was curvilinear in every animal, such that for a given fall in \( P_{\text{ao}} \), the magnitude of rib displacement decreased as pressure was more negative.

On the other hand, the direction of displacement of rib 10 changed with the stimulation frequency. The relationship between rib displacement and \( \Delta P_{\text{ao}} \) computed for each individual animal is shown in Fig. 2A, and the relationship computed for the animal group is shown in Fig. 2B. When the stimulation frequency was set at 10 Hz, the rib moved in the cranial direction in five animals and remained stationary in one animal. However, when frequency was set at 11 Hz, the rib moved cranially in three animals and caudally in three animals, and this trend toward a more caudal displacement continued as frequency increased further. Consequently, when frequency was 15 Hz or greater, the rib invariably moved in the caudal direction. For the six animals, therefore, the rib displacement corresponding to a \( \Delta P_{\text{ao}} \) of 10 cm \( H_2O \) was \( +1.94 \pm 0.55 \) mm, whereas the displacement corresponding to a \( \Delta P_{\text{ao}} \) of 40 cm \( H_2O \) was \( -7.98 \pm 1.32 \) mm (Fig. 2B).

Similar qualitative results were obtained during ramp stimulation of the phrenic nerves, as shown for one representative animal in Fig. 3. In every animal, as the frequency of stimulation and \( \Delta P_{\text{ao}} \) increased, rib 5 gradually progressed in the caudal direction until the cessation of stimulation. In contrast, rib 10 moved first in the cranial direction and then in the caudal direction.

Effect of pneumothorax on rib displacement during phrenic stimulation. When the phrenic nerves were stimulated at 20 Hz in the presence of pneumothorax, rib 5 remained stationary in every animal, and rib 10 remained essentially stationary as well. Thus, whereas the displacement of rib 10 for the six animals before pneumothorax was \( -8.46 \pm 0.67 \) mm, after pneumothorax it was \( -0.31 \pm 0.32 \) mm (\( P < 0.001 \)).

Fig. 1. Relationship between the axial displacement of rib 5 and the change in airway opening pressure (\( \Delta P_{\text{ao}} \)) during isolated stimulation of the C5-C6 phrenic nerve roots with different stimulation frequencies (10–20 Hz) in 6 animals with the airways closed. Lines with different symbols correspond to different animals. Positive values for rib displacement correspond to displacements in the cranial direction. In each animal, stimulation at 10 Hz (data points on the left) produced a caudal rib displacement; as the stimulation frequency increased to 20 Hz (data points on the right), \( \Delta P_{\text{ao}} \) increased and the caudal displacement of the rib increased as well.

Fig. 2. A: relationship between the axial displacement of rib 10 and \( \Delta P_{\text{ao}} \) during isolated stimulation of the C5-C6 phrenic nerve roots with different stimulation frequencies (10 to 20 Hz) in 6 animals. Same symbols and same convention as in Fig. 1. B: mean \( \pm \) SE values of rib 10 displacement obtained from the 6 animals at fixed values of \( \Delta P_{\text{ao}} \). Note that during stimulation at 10 Hz (A, data points on the left) and with a \( \Delta P_{\text{ao}} \) of –10 cm \( H_2O \) (B), the rib moved in the cranial direction. However, as the stimulation frequency and \( \Delta P_{\text{ao}} \) increased, the direction of rib displacement was reversed from cranial to caudal.
Rib displacement during spontaneous, isolated diaphragm contraction (experiment 2). The records of Pao, Pab, and rib displacement obtained in a representative animal during two unimpeded breaths and an occluded breath performed with the diaphragm alone are shown in Fig. 4; the EMG activity in the 2nd left parasternal intercostal muscle is also shown as a time reference. During the inspiratory phase of unimpeded breathing, rib 5 moved in the caudal direction in all animals, whereas rib 10 consistently moved in the cranial direction. At peak inspiration, therefore, the displacement of rib 5 and rib 10 for the seven animals was $-0.72 \pm 0.16$ and $+1.30 \pm 0.10$ mm, respectively. The corresponding value of $P_{ab}$ was $+1.97 \pm 0.24$ cmH$_2$O.

The difference between the displacement of rib 5 and that of rib 10 was amplified during occluded breaths. Thus, as the inspiratory effort against the occluded airway increased and Pao gradually became more negative, rib 5 continuously progressed in the caudal direction (Fig. 4). In contrast, rib 10 continued to move in the cranial direction throughout inspiration, and Fig. 5 shows the relationship between the displacement of the rib and Pao obtained for each individual animal. Although rib 10 tended to stabilize in the last part of inspiration in the two animals that developed the largest $\Delta$Pao, a displacement of the rib in the caudal direction was not observed. For the animal group, the displacement of rib 5 and rib 10 at peak inspiration was $-4.00 \pm 0.48$ and $+1.32 \pm 0.18$ mm, respectively, while $\Delta$Pao was $-13.4 \pm 1.6$ cmH$_2$O.

When the airway was occluded for a series of consecutive breaths, $\Delta$Pao increased progressively with increasing breath number, and the caudal motion of rib 5 increased in amplitude as well. At the end of the run, the displacement of the rib at peak inspiration was $-5.42 \pm 0.50$ mm while $\Delta$Pao was $-20.7 \pm 1.9$ cmH$_2$O. Yet, in each animal, the position of rib 10 at this point was still cranial relative to its relaxation position. As shown in Fig. 6, the rib typically moved cranially in the first third of the inspiratory effort and then plateaued. In four of seven animals, the rib even returned slightly toward its relaxation position at the end of the effort. At no time in no animal, however, was the rib caudal relative to its relaxed FRC position. For the animal group, the net displacement of rib 10 at peak inspiration was $+1.63 \pm 0.23$ mm.

**DISCUSSION**

The first important result of this study is the demonstration, in agreement with our hypothesis, that the displacement of the lower ribs during isolated stimulation of the phrenic nerves depends on the level of diaphragm activation. When the stimulation frequency during rectangular or ramp stimulation was 15–20 Hz and $\Delta$Pao was $-25$ cmH$_2$O or greater, both the upper and the lower ribs moved caudally (Figs. 1–3). This result confirms our previous observation (8, 9). The finding that the caudal displacement of the lower ribs was eliminated during stimulation in the presence of bilateral pneumothoraces also indicates that this displacement is entirely the result of the...
fall in Ppl. However, when the stimulation frequency was 10 Hz, such that ΔPao (and ΔPpl) was approximately −10 cmH₂O, the upper ribs still moved caudally but the lower ribs moved cranially. This cranial displacement is consistent with the inspiratory increase in the diameters of the lower rib cage observed during resting breathing and during phrenic nerve pacing in quadriplegic subjects (4, 13, 26, 28). It is also consistent with the lower rib cage expansion previously observed during selective activation of the costal portion of the canine diaphragm (12); although Ppl in these animals was not measured, the airway was kept open and the increase in lung volume during stimulation was only ~100 ml, thus suggesting that ΔPpl was small. Thus, based on the observations obtained in the first experiment of the current study, one can reasonably explain the apparent discrepancies between previous studies of the action of the diaphragm on the lower rib cage. That is, when activation of the diaphragm is low, as it is the case during quiet breathing in quadriplegic subjects, the muscle causes a cranial displacement of the lower ribs, but when the level of activation increases, as during high frequency stimulation of the phrenic nerves, the muscle fibers shorten more and the zone of apposition decreases so that the lower ribs become gradually exposed to pleural pressure. As a result, the cranial motion of these ribs is eliminated and eventually reversed into a caudal motion.

It was expected that the displacement of the lower ribs during spontaneous, isolated contraction of the diaphragm would also be dependent on the level of muscle activation. The second important result of this study, however, is the observation that during spontaneous diaphragm contraction, the lower ribs never moved in the caudal direction. During both resting, unimpeded breathing, and single occluded breaths, rib 10 moved in the cranial direction in every animal, whereas rib 5 moved in the caudal direction (Figs. 4 and 5). Also, and more important, similar rib displacements were seen after the level of diaphragm activation was enhanced by a series of consecutive occluded breaths and ΔPao was increased to −20 to −30 cmH₂O (Fig. 6); in contrast, when such ΔPao values were developed during stimulation of the phrenic nerves, rib 10 was consistently displaced in the caudal direction.

Because the rib displacements observed during spontaneous diaphragm contraction and during phrenic nerve stimulation were compared at similar ΔPao values, and because these ΔPao values were obtained with no airflow, it is unlikely that the difference in rib displacement is related to a difference in ΔPpl. Also, the maintenance of a cranial rib displacement during spontaneous contraction cannot be attributed to the preservation of an intact parasternal intercostal in the second left interspace. To be sure, the muscle was active during inspiration, and, thus contributed to ΔPao. Therefore, if this parasternal intercostal were also denervated, a given displacement of rib 10 would be associated with a smaller ΔPao, so that the relationship between rib displacement and ΔPao during spontaneous contraction (shown in Figs. 5 and 6) would be displaced to the left. However, measurements of the respiratory effect of the parasternal intercostals in the dog have shown that the ΔPao generated by a maximal, isolated contraction of the muscle in the second left interspace is less than −1.0 cmH₂O (11). Moreover, the activation of the muscle in our animals was probably submaximal, including after a series of occluded breaths. The impact of the muscle on ΔPao, therefore, must be very small, and this implies that the action of the isolated diaphragm on the lower ribs depends not only on the magnitude but also on the mode of muscle activation.

Although the mechanism of the differential displacement of the lower ribs during spontaneous diaphragm contraction vs. phrenic nerve stimulation cannot be identified on the basis of the present measurements, studies in the cat (14, 27) and in the dog (12, 19) have shown a somatotopic projection of the different branches of the phrenic nerve. In the dog, innervation of the dorsal part of the crural region of the diaphragm is provided primarily by a pool of motoneurons situated in the seventh cervical segment (19). However, it is difficult to stimulate the C7 phrenic nerve roots without producing a pneumothorax. Therefore, stimulation of the phrenic nerves in

Fig. 5. Relationship between the axial displacement of rib 10 and ΔPao obtained during single occluded breaths from 7 animals breathing with the diaphragm alone. The shaded area corresponds to the relationship (mean ± SE) obtained during isolated stimulation of the C5-C6 phrenic nerve roots with different stimulation frequencies (data shown in Fig. 2B).

Fig. 6. Relationship between the axial displacement of rib 10 and ΔPao obtained at the end of a series of 15–20 occluded breaths from 7 animals breathing with the diaphragm alone. The shaded area corresponds to the relationship (mean ± SE) obtained during isolated stimulation of the C5-C6 phrenic nerve roots with different stimulation frequencies (data shown in Fig. 2B).
experiment 1 involved the C5 and C6 but not the C7 nerve roots, and this implies that in these animals, the crural portion of the diaphragm may not have been as fully activated as the costal portion. On the other hand, using fluorescent microspheres to measure regional blood flow in the canine diaphragm, Brancatissano et al. (2) and Johnson et al. (17) have shown that during spontaneous breathing in animals with all respiratory muscles intact (i.e., during coordinated contraction of the diaphragm and inspiratory intercostals), the entire diaphragm is activated, including the dorsal part of the crural portion. It is reasonable to speculate, therefore, that in the present study, the relative activation of the crural diaphragm was greater during spontaneous contraction (experiment 2) than during phrenic nerve stimulation (experiment 1). In that case, for a given ∆Pao, the shortening of the costal muscle fibers would be smaller during spontaneous contraction, and the zone of apposition would be larger. The lower ribs, therefore, would be less exposed to the expiratory effect of Ppl, and Pab would be more effective in expanding the lower rib cage (greater appositional force). In other words, even though the crural portion of the diaphragm has no effect on the lower rib cage when it contracts alone (12), it would play an important role during spontaneous diaphragm contraction in allowing the costal portion to lift the lower ribs.

Many studies of the mechanics of the diaphragm have heretofore been performed using isolated, supramaximal stimulation of the C5 and C6 phrenic nerve roots in dogs (1, 7, 9, 16, 25). This technique is useful because it produces a constant, well-defined level of muscle activation and avoids simultaneous contraction of other muscles. Therefore, it allows accurate assessments of the relationships among diaphragm muscle length, diaphragm displacement, ∆Ppl, and ∆Pab to be made. It also allows for the precise evaluation of the response of the muscle to different conditions, such as lung inflation (9, 16, 25) and ascites (20). An important implication of the current study, however, is that observations made during such stimulation of the phrenic nerves do not necessarily reflect the physiological action of the diaphragm.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

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