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Effects of Aminophylline on Regional Diaphragmatic Shortening after Thoracotomy in the Awake Lamb

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Aminophylline has been reported to augment diaphragmatic contraction, although this remains a controversial finding. We studied the effect of aminophylline on regional diaphragmatic shortening, changes in transdiaphragmatic pressure (ΔP_{di}), and integrated regional electromyographic (EMG) activity of the diaphragm (E_{di}) after a right thoracotomy in nine lambs using sonomicrometry, esophageal and gastric balloons, and EMG. Sonomicrometer crystals and EMG leads were implanted into the costal and crural regions of the diaphragm through a right thoracotomy, and a tracheostomy was performed. The animals were studied while awake within 4 days after surgery. Fractional costal and crural diaphragmatic shortening was measured using the sonomicrometer; ΔP_{di} was calculated from esophageal and gastric pressures. Respiratory variables were measured through the tracheostomy. Data were collected during quiet breathing and during CO_2 rebreathing. After control measurements, aminophylline (10 mg/kg) was administered intravenously, producing a serum concentration of 17.7 ± 1.5 μg/ml. Aminophylline did not augment shortening, increase ΔP_{di}, or overcome postoperative diaphragmatic inhibition acutely in the awake sheep after a right lateral thoracotomy. A small decrease of end-tidal CO_2, from 5.2% to 4.9%, was measured at rest during aminophylline infusion, but E_{di} was unchanged. Although during CO_2 rebreathing diaphragmatic shortening increased, the addition of aminophylline did not further augment shortening. Our data in awake lambs suggest that aminophylline does not improve diaphragmatic contraction in the acute postoperative period. (Key words: Pharmacology: aminophylline; theophylline. Muscle, skeletal: diaphragm. Surgery, thoracotomy: postoperative respiratory function; ventilation.)

DIAPHRAGMATIC DYSFUNCTION after thoracic and upper abdominal surgery is believed to be a major cause of the pulmonary complications that can develop after these procedures.[1,2] A recent study by Dureuil and coworkers[3] suggested that aminophylline can partially overcome the inhibition of human diaphragmatic function that occurs after upper abdominal surgery. This study measured an indirect parameter of global diaphragmatic function, transdiaphragmatic pressure (P_{di}). However, Dureuil and coworkers concluded that the increases of P_{di} they measured after aminophylline administration could be due to a positive inotropic effect on the diaphragm itself, to changes in its resting length, or to alterations of central respiratory stimulation, or a combination of these factors. Without the simultaneous determination of diaphragmatic activation (the integrated electromyogram [EMG] of the diaphragm [E_{di}]) and diaphragmatic muscle length, one cannot assess contractility (the amount of force generated for a given degree of activation, *i.e.*, P_{di}/E_{di}) or the length–force characteristics of the diaphragm at a given point on its length–tension curve.[4]

Controversy exists as to whether methylxanthines such as aminophylline can augment the contractility of the diaphragm. A positive inotropic effect has been inferred from *in vitro* studies of isolated diaphragmatic muscle preparations[5,6] and *in vivo* studies of P_{di} and EMG recordings.[7,8] However, several studies have reported no increase of diaphragmatic contractility with aminophylline,[9–12] and, therefore, whether aminophylline administration increases diaphragmatic contractility in the intact animal remains uncertain.

We undertook this study to examine the effect of therapeutic intravenous doses of aminophylline on diaphragmatic function in an awake animal after a thoracotomy, a model we have developed in our laboratory.[13] We studied nine lambs within 4 days after a thoracotomy, when inhibition of diaphragmatic function is known to be present,[13,14] and used recently developed methods to examine some of the questions left unanswered by previous investigations of aminophylline. Sonomicrometry was used to measure regional diaphragmatic length and shortening directly, EMG electrodes to measure regional E_{di}, and balloon catheters to measure ΔP_{di}, thereby allowing us to assess regional diaphragmatic contractility, length–force and force–velocity characteristics, and neural activation. Sonomicrometry offers a unique way of directly examining regional diaphragmatic shortening and length in awake animals.[13,15] Pairs of piezoelectric crystals are implanted between diaphragm muscle fibers, and one of the pair is stimulated to emit sound waves. The distance between the crystals is then continuously determined by measuring the time taken for the emitted sound wave to travel from the transmitting to the receiving crystal. One can thereby continuously measure the precise fractional shortening of the costal and crural diaphragmatic regions during each breath.

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We hypothesized that if aminophylline augmented diaphragmatic contractility, then either an increased shortening fraction or an increased ΔP_{di} or both would be measured at any given level of E_{di}. In addition, no change in the diaphragm's resting length should be measured, because this factor alone will alter the tension developed by the diaphragm. In this manner we could more accurately assess the mechanisms of the effect of aminophylline on the diaphragm. In this study we used aminophylline (theophylline ethylenediamine), the intravenous preparation of theophylline, equivalent to 85% anhydrous theophylline by weight. Serum concentrations were measured, as is the convention in clinical medicine, as serum theophylline, in micrograms per milliliter.

Materials and Methods

Implantation

All experiments were approved by the Subcommittee on Animal Studies of the Massachusetts General Hospital. Ten Suffolk lambs, 4–5 months old and weighing 29–32 kg (average 30.8 kg) underwent a right lateral thoracotomy through the ninth intercostal space under general endotracheal anesthesia while breathing 2–2.5% halothane in O_2. Using sterile procedures, the costal and crural portions of the diaphragm were exposed and a pair of sonomicrometer crystals (Dimension 3, Hacienda Heights, CA) were implanted between muscle fibers in each region, spaced an average of 15 mm apart. The crystals were mounted on 2-mm stalks of PE190 tubing and attached to 15-mm woven Dacron patches with room temperature–vulcanizing silicone rubber (3M Co., St. Paul, MN) by a technique previously described.[13] The costal crystals were implanted on a planar area of the diaphragm over the liver in the anterior axillary line. Care was taken to ensure that the crystals were oriented parallel to one another and perpendicular to the muscle fibers. Proper crystal orientation was confirmed by visualization of the sonomicrometer signal on an oscilloscope (model 5441, Tektronix, Beaverton, OR) prior to closure of the chest.

Corresponding EMG electrodes were implanted adjacent to the crystals, taking care that the regional blood supply was not compromised. EMG wires in the first four animals were constructed of silver-plated copper wire; subsequent implantations were performed with Teflon-coated multistranded stainless steel wires (Medwire 316 SS 7/44T, Mt. Vernon, NY). All of the wire leads were tunnelled subpleurally and subcutaneously and brought out through separate skin incisions. Before closure of the chest, the pleural surface of the diaphragm was stimulated directly with a supramaximal tetanic stimulus at 100 Hz (Microstim, NeuroTechnology, Houston, TX) to assess the maximal attainable shortening of both segments.

The chest was evacuated with a thoracostomy tube (8 mm OD) at the time of closure, and a chest radiograph was taken to confirm the lack of any residual pneumothorax. Local infiltration with 0.5% bupivacaine was administered for postoperative analgesia. A tracheostomy was performed, and the animal was allowed to recover overnight. All implantations were performed by one thoracic surgeon (JCW) using the same techniques. Animals received intramuscular antibiotics (Combiotic, Pfizer, NY, NY) daily after surgery.

Experimental Protocol

The animal model is depicted in figure 1. Lambs were studied 1–4 days after surgery. Previous investigations using this model have demonstrated that there is no change in the degree of diaphragmatic inhibition in the first postoperative week.[13] They were not fed for 24 h prior to each study to avoid gastric distension but were allowed unlimited access to water. All studies were performed with the animals standing awake and unsedated.

A cuffed 7-mm tracheostomy tube was inserted and inflated. Topical nasal anesthesia with 4% lidocaine jelly was administered, and a balloon-tipped catheter (National Catheter Co., model 85842, Mallinkrodt, Argyle, NY) was passed through each nare. One catheter was paced in the stomach and one in the lower third of the esophagus. The gastric balloon was inflated with 3 ml air and the esophageal balloon with 1 ml air. The esophageal catheter's position was confirmed by measuring large negative pressures produced by transient tracheal occlusion and by observing transmitted cardiac pressure artifacts. The catheters were connected to differential pressure transducers (Validyne MP45), and ΔP_{di} was calculated as the difference between changes in gastric pressure (ΔP_{gas}) and esophageal pressure (ΔP_{es}). Airway flow was sensed at the tracheostomy with a Fleisch pneumotachograph (Hans Rudolph model 3700, Kansas City, MO). The signal was integrated to obtain tidal volume and the

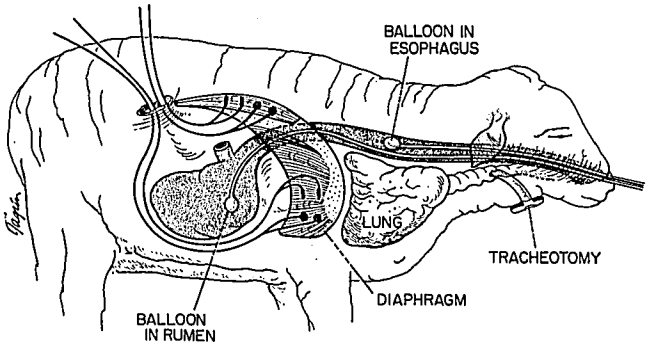

FIG. 1. Lamb model for assessing diaphragmatic function after thoracotomy. The drawing depicts sonomicrometer crystals and electromyography electrodes in the costal and crural regions of the diaphragm. (Reproduced from Torres *et al.*,[15] with permission.)

volume measurement calibrated with a 1-l giant syringe. The EMG signals were amplified (Grass Instruments, Quincy, MA), band-pass–filtered (30 Hz to 3 kHz) and processed through a Paynter filter with a 0.2-s time period. Urine output was collected from the Babraham cage and measured throughout the experiment to provide an additional index of the effect of aminophylline. The presence of any excitatory neurobehavioral effects (tremulousness, agitation) was noted before and after the administration of aminophylline.

Dynamic measurements of the distance between crystals were continuously obtained with a sonomicrometer (model 120, Triton Technology, San Diego, CA).

Expired gas was sampled continuously, and end-tidal CO_2 concentration (FET_{CO_2}) measured with an infrared analyzer (Beckman LB-2, Sensormedics, Anaheim, CA). This was calibrated prior to each study using ambient air and 5% CO_2 in O_2. All data were recorded on an eight-channel recorder (Hewlett-Packard 7758A, Palo Alto, CA).

Lambs first breathed air through the pneumotachograph for several minutes until a steady state was achieved. After this period of quiet breathing, a 5-l reservoir bag containing 5% CO_2 and 95% O_2 was attached to the tracheostomy, and the animal rebreathed CO_2 up to 10% FET_{CO_2}. After CO_2 rebreathing, the lamb recovered for 20–30 min. Two complete control studies were performed in each lamb. The transducers, capnograph, and pneumotachograph were recalibrated prior to each study.

Aminophylline 10 mg/kg was infused over 20 min *via* a percutaneously placed 18-G jugular venous cannula. After this loading dose, an infusion of 0.8 $mg \cdot kg^{-1} \cdot h^{-1}$ was begun, and the serum theophylline concentration was measured 40 min later using fluorescent polarization immunoassay. This dose was demonstrated in pilot studies to produce a serum theophylline concentration of 15–20 μg/ml. The experimental protocol of obtaining respiratory measurements during quiet breathing and CO_2 rebreathing was then repeated twice during the aminophylline infusion.

Data were measured directly from the strip charts. Five consecutive breaths were measured during quiet breathing and at each 0.5% increment of FET_{CO_2} from 7% to 10%. Timing of the respiratory cycle in all channels, with the exception of FET_{CO_2} (due to the time lag in the signal), was referenced to the pneumotachograph, whose signal most accurately reflected the instant of peak inspiration and end expiration (fig. 2). Raw data were entered into a computer, corrected for the appropriate units of measurement, and analyzed using paired *t* tests, and are presented as mean ± SEM. A *P* value of less than 0.05 was considered significant. Regional diaphragmatic shortening was measured in millimeters and also calculated as the percentage of shortening from resting length at functional residual capacity. E_{di} is expressed in arbitrary units, normalized to the maximal integrated EMG activity measured during the control period (before aminophylline administration) when FET_{CO_2} was 10%. ΔP_{di} was calculated from the difference between the simultaneous changes in P_{gas} and P_{es} measured from end expiration to peak inspiration and is reported in cmH_2O. Above 9% FET_{CO_2}, where expiratory ΔP_{gas} obscured the trace of inspiratory ΔP_{gas}, the inspiratory ΔP_{gas} was assumed to be zero, which may have slightly underestimated ΔP_{di} (fig. 2).

Results

Of the ten lambs who underwent sonomicrometer crystal implantation, one developed an empyema on the second postoperative day and was excluded from the analysis. The remaining nine animals formed the study population. Two of the nine lambs had malfunctioning EMG electrodes, and one had misaligned crural sonomicrometer crystals; the remainder of the implanted measuring devices functioned well.

A serum theophylline concentration within the accepted therapeutic range for bronchodilation of humans[14] was measured in all lambs (17.7 ± 1.5 μg/ml). Seven of nine animals evidenced a brisk diuresis within 0.5 h after the drug loading dose, and all lambs exhibited tremulousness and agitation during but not before aminophylline administration.

Sonomicrometric measurements and cavitary pressures are presented in figure 3. During quiet breathing, costal and crural shortening fractions were 6.3 ± 0.9% and 4.7 ± 1.1% respectively and increased to 18.4 ± 2.1% and 7.1 ± 1.2% during CO_2 rebreathing at 10% FET_{CO_2}. Neither regional shortening nor resting diaphragmatic segment length at functional residual capacity was significantly changed after administration of aminophylline. Although costal shortening during the control period tended to decrease at FET_{CO_2} greater than 9.5%, these changes were not statistically significant. ΔP_{di}, ΔP_{es}, and ΔP_{gas} showed no changes from control after aminophylline infusion. Inspiratory ΔP_{gas} remained unchanged during CO_2 rebreathing and never exceeded +2 cmH_2O. Expiratory increases in ΔP_{gas}, denoting recruitment of abdominal muscles, developed at an FET_{CO_2} of 8–8.5%. E_{di} did not change during aminophylline infusion, nor did the ratio of $\Delta P_{di}/\Delta E_{di}$, which is the slope of the plot of ΔP_{di} *versus* ΔE_{di} in the costal and crural regions (fig. 4).

Respiratory measurements are presented in table 1. The FET_{CO_2} during quiet breathing was slightly less during aminophylline administration than during control (4.9 ± 0.2% *vs.* 5.2 ± 0.1%). A tendency toward a small increase of respiratory rate was measured during aminophylline administration; this approached, but did not reach, statistical significance (26 *vs.* 23 breaths/minute, *P*

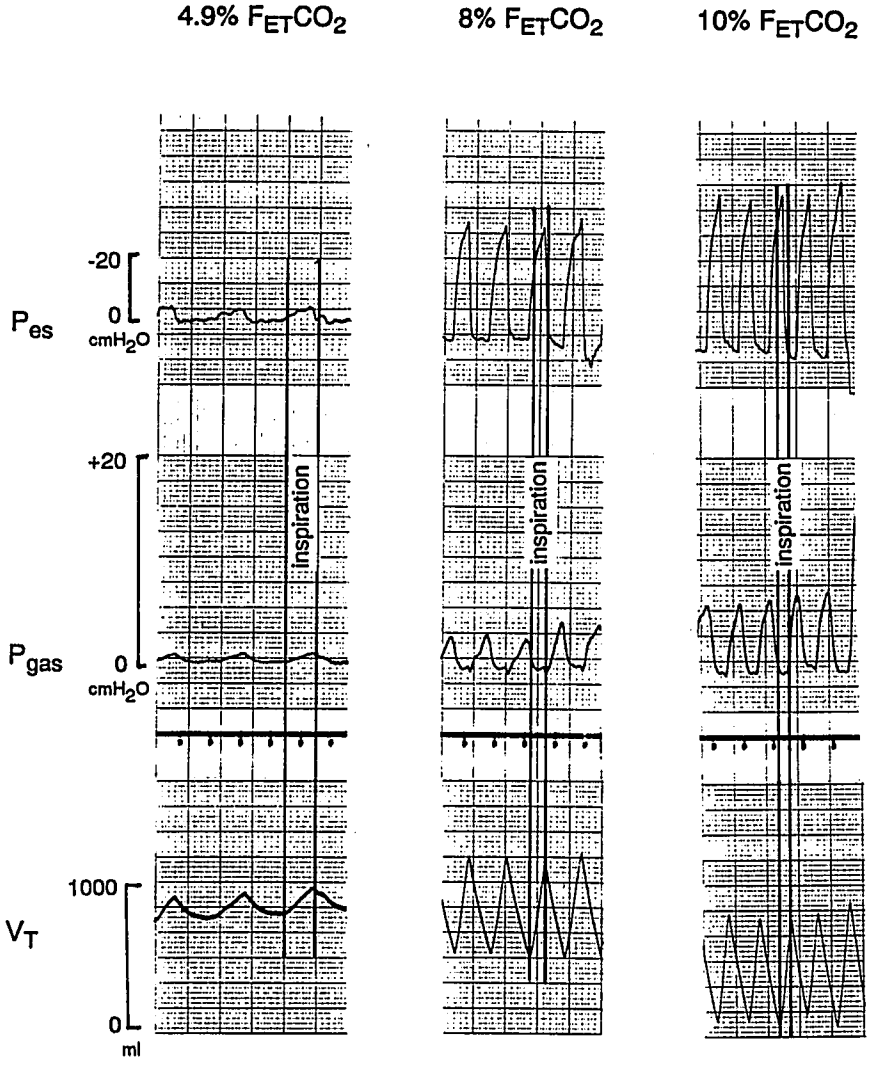

FIG. 2. Representative traces of esophageal pressure (P_{es}), gastric pressure (P_{gas}), and airway flow at resting breathing and at 8.0% and 10% $F_{ET}CO_2$. The onset of expiratory airway flow coincides with the onset of the rise in P_{gas}, denoting recruitment of expiratory abdominal muscles (arrows). At 10% $F_{ET}CO_2$, the large expiratory pressure wave obscures the trace of inspiratory P_{gas}.

= 0.08). Tidal volume and minute ventilation both at rest and during CO_2 rebreathing were unchanged by aminophylline treatment.

Discussion

A clinically therapeutic serum concentration of aminophylline did not alter costal or crural diaphragmatic function in the awake lamb after a thoracotomy. CO_2 rebreathing increased regional diaphragmatic shortening, ΔE_{di}, and ΔP_{di}, but treatment with aminophylline did not further augment these increases. Our lamb model of thoracotomy assesses four parameters necessary to define the mechanism of regional changes of diaphragmatic contractility: regional diaphragmatic activation (E_{di}), global force generation (P_{di}), regional diaphragmatic length (shortening fraction and resting length), and respiratory cycle timing.[4] Not all of this information was available in previous investigations of aminophylline on the diaphragm. As Dureuil and coworkers concluded in their study, the increased P_{di} they measured after aminophylline administration to patients after cholecystectomy could be attributed to several mechanisms, including improved diaphragmatic contractility, decreased reflex neural inhibition, or increased central respiratory drive.[3] Our awake lamb model provided us with the ability to make some distinction between the effect of aminophylline on diaphragmatic muscle itself and on neural input as estimated by E_{di}.

The methodology used here assumes that 1) E_{di} is representative of the activation of the entire diaphragm; 2) during submaximal stimulation, E_{di} increases proportionally to the level of neural input; 3) E_{di} is proportional to P_{di} at submaximal levels of activation; and 4) all respiratory muscle groups maintain a similar proportional contribution to ventilation with and without aminophylline. These

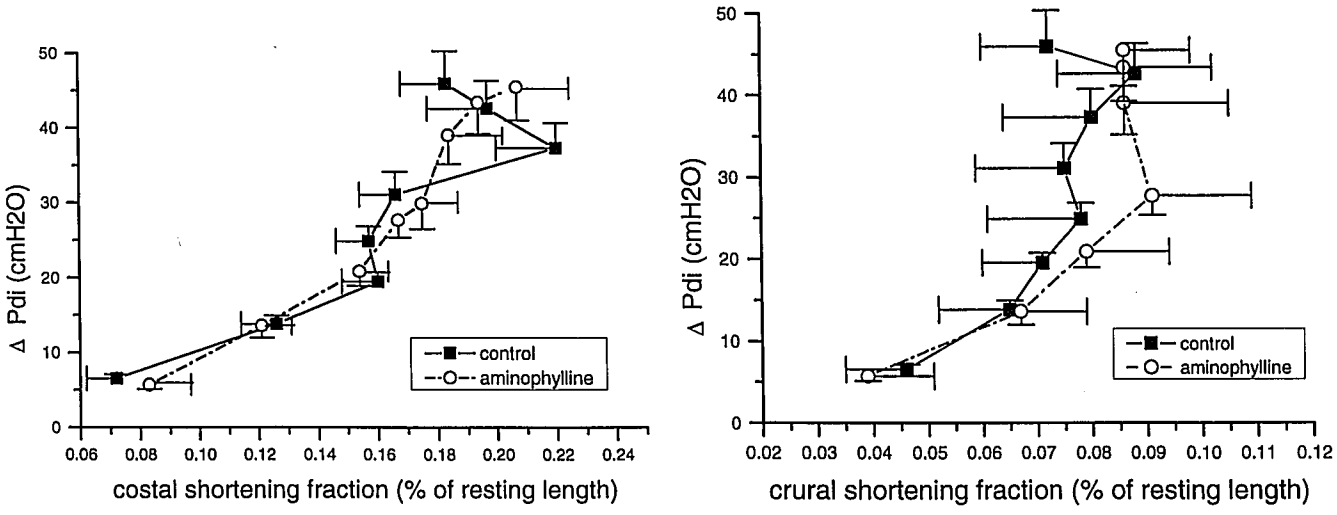

FIG. 3. Changes in transdiaphragmatic pressure (ΔP_{di}) and segmental shortening of the costal (*left*) and crural (*right*) regions of the diaphragm as measured by sonomicrometry in nine (costal) and eight (crural) lambs. Filled squares denote control values, and open circles denote values during aminophylline therapy. All measurements were made at quiet breathing and from 7 to 10% FET_{CO_2} in 0.5% increments. There is no significant difference after administration of aminophylline in any of the measurements.

assumptions have some limitations. Because tetanic stimulation was not used, the complete activation of the diaphragm or maximal ΔP_{di} could not be assessed. One cannot know, therefore, that at maximal activation, aminophylline does not improve contractility. The measurement of E_{di} during eupneic breathing is a reflection of diaphragmatic activation but may not have a truly linear relationship with motor unit recruitment, although recent data suggest that a reasonably linear relationship does indeed exist during spontaneous ventilation under conditions of constant proportional activation of the various respiratory muscles.[16] In addition, the measure of ΔP_{di}/ΔE_{di} under these conditions assumes that the motor units sampled by the EMG electrodes are representative of the response of the diaphragm as a whole. The three fiber types of the diaphragm (fast-twitch oxidative glycolytic, fast-twitch glycolytic, and slow-twitch oxidative) and their varying innervations might be affected differently by aminophylline. This study, however, examined whether aminophylline is a useful therapeutic agent in the intact animal after thoracotomy under clinical conditions. There is no alternative technique that can address this question without these limitations, and there is no evidence in the literature that the drug exerts a differential effect.

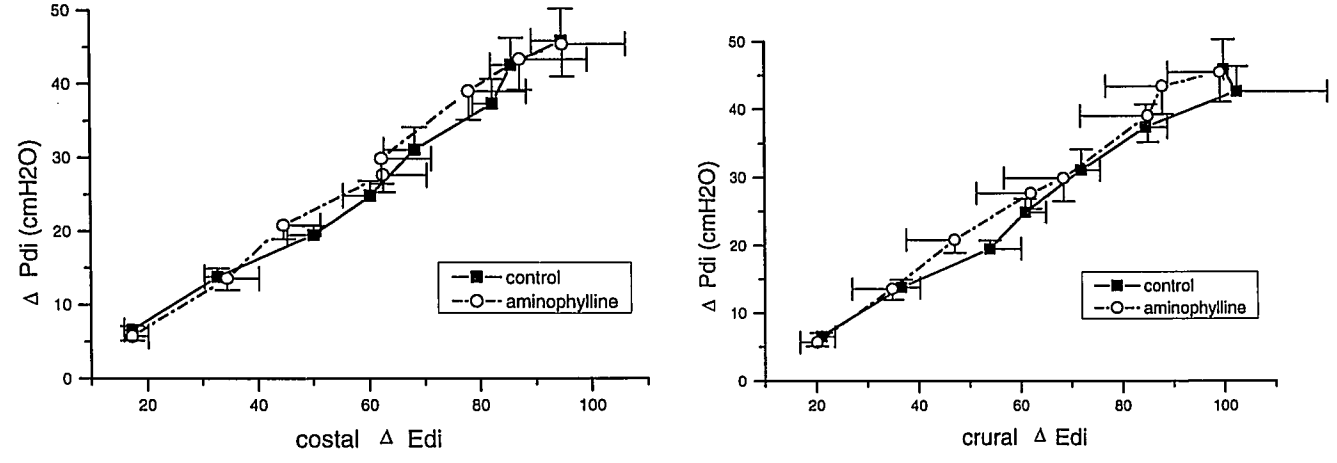

FIG. 4. Ratio of transdiaphragmatic pressure change (ΔP_{di}) to change in costal (*left*) and crural (*right*) regional diaphragmatic electromyogram (ΔE_{di}) in seven lambs. ΔE_{di} is expressed in arbitrary units derived by normalizing the measured values to the electromyographic signal at 10% FET_{CO_2} during the control study. Symbols are the same as in figure 3.

TABLE 1. Respiratory Variables: Postoperative Days 1–4

	Quiet Breathing		10% FET_{CO_2}	
	Control	Theophylline	Control	Theophylline
V_T (ml/min)	244 ± 20	190 ± 23	887 ± 64	912 ± 79
Minute ventilation (l/min)	5.2 ± 0.43	4.8 ± 0.44	42.3 ± 1.7	41.5 ± 2.3
Respiratory rate (breaths/min)	23.3 ± 0.8	25.9 ± 1.4	49.2 ± 2.4	47.6 ± 2.7
T_I (s)	0.91 ± 0.05	0.82 ± 0.06	0.58 ± 0.05	0.61 ± 0.05
T_E (s)	1.72 ± 0.11	1.58 ± 0.13	0.68 ± 0.04	0.71 ± 0.05
V_T/T_I (ml/s)	253 ± 21	235 ± 20	1,549 ± 60	1,504 ± 67
V_T/T_E (ml/s)	224 ± 11	190 ± 16	887 ± 41	912 ± 52
FET_{CO_2} (%)	5.2 ± 0.1	4.9 ± 0.2*	10	10

V_T = tidal volume; T_I = inspiratory time; T_E = expiratory time. * $P < 0.05$ by paired t test.

Contractility can be defined as the force (P_{di}) produced by a given amount of activation (measured as E_{di}).[10] If the P_{di}/E_{di} ratio increases, the contractility of the diaphragm is assumed to have increased, although the mechanisms causing this increase can vary. For example, the diaphragm, like any other muscle, will generate differing amounts of force for a given level of stimulation, depending on its resting length. There is an optimal point along this length–tension curve at which each muscle operates most efficiently; that is, the greatest amount of force will be generated at the same level of excitation. One must therefore be able to determine that the segmental resting length has not changed, or be able to control for that change when comparing P_{di} levels measured at different resting lengths. In addition, altered resting length may alter the size of the zone of apposition, the part of the diaphragm that lies apposed to the rib cage. Changes in the size of this zone will alter the complex interactions between the diaphragm and the rib cage and abdomen, and can make comparisons of P_{di} before and after such a change inaccurate and potentially misleading. The velocity of shortening of the diaphragm will also alter contractility. The same fractional shortening achieved at a slower rate will generate greater force, even though the diaphragm's motion and level of neural input are similar. Because the velocity of shortening is determined by inspiratory time when shortening is constant, an increase of respiratory rate, if it results from a decreased inspiratory time, will increase mean velocity and decrease P_{di}, and thereby decrease contractility. Finally, there may be alterations of the inotropic state of the muscle itself. This can be inferred if $\Delta P_{di}/\Delta E_{di}$ increases while the resting length and velocity of shortening are unchanged.

A 0.3% reduction in FET_{CO_2} ($P < 0.05$) was measured during aminophylline treatment. Despite this reduction, the minute ventilation was unchanged, and the precise mechanism behind the decrease is unknown. Although a small increase of respiratory rate was measured during aminophylline infusion, this change fell short of statistical significance, and tidal volume was not increased. There was no increase in E_{di}, and thus contractility ($\Delta P_{di}/\Delta E_{di}$) was unchanged because the amount of neural activation with each breath was unchanged. Theophylline is known to affect the medullary respiratory centers and is a central respiratory stimulant.[17] It is used as a therapeutic agent to treat apnea of prematurity, where stimulation occurs at serum concentrations of 5–10 μg/ml. It is possible that increases of P_{di} reported in previous studies of intact animals may have a similar mechanism of central respiratory stimulation.

In this study we used sonomicrometry to make direct and continuous measurements of regional diaphragmatic shortening in an awake, intact lamb. Sonomicrometry has been shown to provide an accurate method of measuring diaphragmatic segment length and has allowed important information not obtained by earlier investigations to be measured with consistency and precision.[13,15] There are, however, several potential technical problems with using this technique that must be solved for each successful application. There must always be accurate crystal alignment and stabilization so that artifact does not interfere with measurement of the true maximum and minimum excursions. We have devised a method of mounting, aligning, and stabilizing the crystals that results in accurate placement. In addition, measurements should be made during and after implantation to guide the surgeon and confirm alignment. There are two regions of the diaphragm, each with its own distinct anatomy and actions.[18] The costal region, with insertions on the ribs, moves the rib cage outward, causing inspiratory activity. The crural region has no direct connection to the ribs and may act to decrease the area of the zone of apposition or to adjust muscle tension. For this reason we placed sonomicrometers in both regions of the diaphragm and ensured that the crystals were implanted away from the zone of apposition to minimize adhesions to the chest wall and over the liver to minimize the changes in shape that might otherwise be measured. Last is the concern that implantation itself may alter diaphragm function or injure the muscle. In a previous study we performed histologic

examinations of tissue samples from diaphragm at and between sonomicrometer implantation sites.[13] Only a 0.6-mm zone of fibrosis surrounding each chronically implanted crystal was noted. Normal stimulated tetanic shortening fractions were consistently measured at the time of implantation, and throughout the 28-day postthoracotomy study period in our previous experiments, arguing against alterations in diaphragm function due to implantation.

Possibly the dose of aminophylline used in our study was inadequate to cause an increase in diaphragmatic shortening. Although it is difficult to state with certainty what the maximal safe serum theophylline concentration is for lambs, several factors lead us to conclude that the commonly used human value (20 μg/ml) is a valid upper limit in our lamb model and that higher concentrations are poorly tolerated. Aminophylline is a mild diuretic, and seven of our nine animals had a marked diuresis after the aminophylline infusion. Tremulousness and agitation, common effects seen at high serum levels in humans, were observed in all our lambs. In one animal we administered an additional loading dose of 5 mg/kg, producing a serum concentration of 32 μg/ml. In this lamb, tremulousness and agitation was pronounced, similar to a human whose theophylline concentration is in the toxic range, but again no improvement was noted in diaphragmatic shortening. We believe that the toxic threshold of this drug is similar in lambs and humans and that the therapeutic window is limited to serum concentrations less than 20 μg/ml because of the excessive central nervous system stimulation that develops at higher concentrations.

Some studies on the effect of aminophylline on isolated diaphragm strips have measured an increase in isometric contractile force, but several factors caution against the extrapolation of *ex vivo* muscle bath studies to the *in vivo* situation. In the intact animal, the diaphragm works in concert with the chest wall and accessory muscles as an integrated system, controlled by the medullary respiratory center. Most isolated muscle preparations have used theophylline or aminophylline doses that are far in excess of those tolerated by a live preparation,[19,20] although Viires and colleagues suggested that in the usual muscle bath preparation, inadequate intracellular drug concentrations are achieved if the bath contains a drug concentration equal to that measured in blood.[5] Several bath studies have been conducted at room temperature[19,20]; increases in the contractility of muscle strips *in vitro* are known to occur at decreasing temperature.[21] Reid and Miller[9] recently addressed the issue of theophylline and the augmentation of maximal force generation and measured no increases in their *in vitro* preparation; endurance was also reduced and recovery inhibited. They suggested that neural effects, especially increased central respiratory drive, could account for the augmentation of diaphragmatic strength with theophylline reported in situations of limited inspiratory drive in intact animals or humans.[9]

Our findings in lambs differ from those of a number of clinical studies. Some examined diseased adult humans, who may be physiologically quite different from our awake lamb, because of variations of species, age, and presence of pulmonary parenchymal and airway disease, as well as superimposed diaphragmatic fatigue. Supinski and colleagues studied caffeine and theophylline in normal adult volunteers using P_{di}, E_{di}, and inductance plethysmography, and reported increases in P_{di} without increases in E_{di},[22] and Aubier and colleagues reported similar results in their investigation of healthy adults.[23] It is difficult, however, to compare such studies to the very different situation of acute diaphragmatic dysfunction after surgery, which appears to be neurally mediated. Moreover, numerous other investigators have been unable to confirm the positive findings of those studies.[10,11,24]

More pertinent to our study, Dureuil and colleagues specifically examined the effect of aminophylline infusion on diaphragmatic function in the postoperative period.[3] In their investigation, eight patients who underwent a cholecystectomy were studied, using P_{di} to assess global diaphragmatic function. Also calculated was the diaphragmatic index,[25] which separates the abdominal pressure (P_{gas}) component of P_{di} and assumes that any contribution of P_{gas} is due solely to descent of the diaphragm. Dureuil and colleagues found that intravenous administration of 6 mg/kg aminophylline 6 h after a cholecystectomy resulted in a 50% return of the diaphragmatic index to the value measured prior to surgery. Several factors may account for the differing results in our lamb study. The standing posture of the sheep and the conformation of its rib cage are quite different from the human; these can effect respiratory mechanics of both the diaphragm and the rib cage. Species differences in the diaphragm's susceptibility to an inotropic effect of aminophylline are possible. Diaphragmatic function was inferred by Dureuil *et al.*[3] from P_{di} alone, whereas in our study we also measured E_{di}, segment length and shortening, and respiratory variables. It is important to note that respiratory rate increased in Dureuil *et al.*'s study from 16 to 20 breaths/minute,[3] suggesting that central mechanisms may have contributed to their results. Although in our study we examined the acute effects on diaphragmatic function of a thoracotomy rather than upper abdominal surgery, both upper abdominal and thoracic surgery have been reported to elicit similar inhibition of diaphragmatic function in animals[13,26] as well as in humans.[2]

Our measurements in the awake lamb suggest that aminophylline at therapeutic serum concentrations (10–20 μg/ml) does not act as an inotropic agent upon the diaphragm, nor does it improve diaphragmatic function by

other mechanisms. Aminophylline's effects on the central respiratory center may have caused the respiratory changes measured in our study and may have contributed to increases of P_{di} reported by other investigators.[3,7,8] Further corroboration of these results in humans is needed to determine if important species differences exist in aminophylline's mechanism of action.

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