Mechanical ventilation results in progressive contractile dysfunction in the diaphragm

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Received 27 August 2001; accepted in final form 28 December 2001

Mechanical ventilation results in progressive contractile dysfunction in the diaphragm. J Appl Physiol 92: 1851–1858, 2002; 10.1152/japplphysiol.00881.2001.—These experiments tested the hypothesis that a relatively short duration of controlled mechanical ventilation (MV) will impair diaphragmatic maximal specific force generation (specific $P_o$) and that this force deficit will be exacerbated with increased time on the ventilator. To test this postulate, adult Sprague-Dawley rats were randomly divided into one of six experimental groups: 1) control ($n = 12$); 2) 12 h of MV ($n = 4$); 3) 18 h of MV ($n = 4$); 4) 18 h of anesthesia and spontaneous breathing ($n = 4$); 5) 24 h of MV ($n = 7$); and 6) 24 h of anesthesia and spontaneous breathing ($n = 4$). MV animals were anesthetized, tracheostomized, and ventilated with room air. Animals in the control group were acutely anesthetized but were not exposed to MV. Animals in two spontaneous breathing groups were anesthetized and ventilated for either 18 or 24 h. No differences ($P > 0.05$) for MV resulted in a reduction ($P < 0.05$) in diaphragmatic specific $P_o$ between control and the two spontaneous breathing groups. In contrast, compared with control, all durations of MV produced a reduction ($P < 0.05$) in diaphragmatic specific tension at stimulation frequencies ranging from 15 to 160 Hz. Furthermore, the MV-induced decrease in diaphragmatic specific $P_o$ was time dependent, with specific $P_o$ being $18\%$ and $46\%$ lower ($P < 0.05$) in animals mechanically ventilated for 12 and 24 h, respectively. These data support the hypothesis that relatively short-term MV impairs diaphragmatic contractile function and that the magnitude of MV-induced force deficit increases with time on the ventilator.

respiratory muscles; muscle atrophy; weaning from mechanical ventilation; muscle injury

MECHANICAL VENTILATION (MV) is used clinically to sustain ventilation in patients who are incapable of maintaining adequate alveolar ventilation on their own. Respiratory failure, neuromuscular diseases, drug overdoses, and recovery from general anesthetics are common indications for the use of MV (11). Problems in weaning patients after prolonged MV have been reported to occur in a significant number of patients (3, 13, 24). It is postulated that a frequent cause of difficult weaning is respiratory muscle failure that occurs because of inspiratory muscle (e.g., diaphragm) force and endurance defects (3, 14, 23). Therefore, understanding the effects of prolonged MV on respiratory muscle contractile function is important.

To date, only two published reports have addressed the effects of MV on diaphragm contractile performance in animals. Specifically, Anzueto et al. (1) demonstrated that 11 days of controlled MV in baboons resulted in a decrease in both maximal diaphragmatic force production ($P_o$) and endurance. Another investigation used a rat model and examined the effects of 48 h of MV on the contractile properties of the diaphragm (15). A significant reduction ($\sim 60\%$) in maximal diaphragmatic specific force (specific $P_o$, i.e., force per cross-sectional area) was reported after MV.

Collectively, the aforementioned studies indicate that prolonged MV impairs diaphragmatic contractile function. However, the time course of MV-induced diaphragmatic contractile dysfunction is unknown. This is significant because an understanding of the time course of MV-induced diaphragmatic dysfunction might provide clues into the mechanism(s) responsible for this phenomenon. Specifically, identification of the time course of MV-induced diaphragmatic contractile dysfunction would set the stage for future experiments aimed at determining which biochemical alterations may be responsible for the observed contractile dysfunction. Therefore, these experiments examined the temporal pattern of MV-induced contractile dysfunction in the rat diaphragm. On the basis of preliminary experiments in our laboratory, we hypothesized that as few as 12 h of MV would result in diaphragmatic contractile dysfunction and that the magnitude of MV-induced contractile dysfunction increases with time on the ventilator.
METHODS

Experimental Animals

These experiments were approved by the University of Florida Animal Use Committee and followed the guidelines for animal experiments established by the National Institutes of Health. Healthy, female young adult (4-mo-old) Sprague-Dawley rats were individually housed and fed rat chow and water ad libitum and were maintained on a 12:12-h light-dark cycle for 3 wk before initiation of these experiments.

Preliminary Experiment

Before performing our primary experiments, we conducted a preliminary experiment that was designed to determine whether the diaphragm is mechanically active during controlled MV. Briefly, we placed wire electrodes in the costal diaphragm of four animals and measured electromyograph activity during 24 h of controlled MV (all breaths delivered by the ventilator). No electrical activity in the diaphragm was observed in any of these animals during the 24 h of MV. These results agree with the previous work of Le Bourdelles et al. (15) and support the idea that diaphragmatic contractions do not occur in anesthetized rats during prolonged, controlled MV.

Experimental Design for Primary Experiments

To examine the time course of MV-induced contractile dysfunction in the diaphragm, animals were randomly assigned to one of six experimental groups (Fig. 1).

Protocol for control animals. The animals in the control group were free of intervention before measurement of diaphragmatic contractile properties. That is, these animals were not mechanically ventilated or exposed to long-term anesthesia before study. At the specified time, animals in the control group received an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). After a surgical plane of anesthesia was achieved, the diaphragm was rapidly removed for measurement of in vitro contractile properties.

Protocol for anesthetized and spontaneously breathing animals. To determine whether long-term anesthesia (i.e., pentobarbital sodium) impairs diaphragmatic contractile properties, our experimental design included two groups of anesthetized and spontaneously breathing animals. Diaphragmatic force production in one group of animals (n = 4) was studied after 18 h of spontaneous breathing, whereas the second group (n = 4) was studied after 24 h of spontaneous breathing. Animals in both of these groups were anesthetized and tracheostomized, and a surgical plane of anesthesia was maintained throughout the experimental period by repeated doses of pentobarbital sodium (~10 mg/kg approximately every hour). These animals were not mechanically ventilated and breathed spontaneously during this time. Arterial catheters were placed in the carotid arteries, and arterial blood samples (~100 μl/sample) were removed at 1, 12, and 18 h from each experimental group. Arterial blood samples obtained from animals in the 18-h experimental group were analyzed for pH and blood-gas status by using a blood-gas analyzer (model 1610, Instrumentation Laboratories). Blood samples were not analyzed in the 24-h experimental group because of technical problems. Body temperature was maintained at ~37°C by use of a recirculating heating blanket. Animals in both of these groups were constantly monitored by a technician and exposed to continuing care. Continuing care during anesthesia in both of these experimental groups included expressing the bladder, removing upper airway mucus, lubricating the eyes, rotating the animal, and passively moving the limbs. This care was maintained throughout the experimental period at 2-h intervals. Furthermore, both heart rate and blood pressure (tail cuff procedure) were measured at 2-h intervals throughout the experiment.

Finally, to reduce airway secretions, animals in both spontaneous breathing groups received intramuscular injections of glycopyrrolate (0.04 mg/kg) every 2 h.

Throughout the period of spontaneous breathing, animals received enteral nutrition from the AIN-76 rodent diet with a nutrient composition of 15% proteins, 35% lipids, 50% carbohydrates, and vitamins and minerals (Research Diets, Brunswick, NY). Our planned feeding schedule was designed to provide an isocaloric diet with the nutrients administered every 2 h via a gastric tube; the total daily administration of 69 ml is equivalent to 69 kcal/day. Body fluid homeostasis was maintained via the administration of 2.0 ml·kg⁻¹·h⁻¹ intravenous electrolyte solution. On completion of the prescribed time period (i.e., 18 or 24 h), a segment of the costal diaphragm was removed for in vitro measurement of contractile properties.

Protocol for mechanically ventilated animals. All surgical procedures were performed by using aseptic technique. Animals randomly selected for MV were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). After a surgical plane of anesthesia was reached, the animals were tracheostomized and mechanically ventilated using a volume-driven small-animal ventilator (model CIV-101, Columbus Instruments, Columbus, OH). All breaths were delivered by the ventilator; hence, this mode of ventilation (i.e., controlled MV) results in complete diaphragmatic inactivity. The tidal volume was established at ~1 ml/100 g body wt with a respiratory rate of 80 breaths/min; this respiratory rate was selected to mimic the breathing frequency of adult rats at rest. Additionally, positive end-
expiratory pressure of 1 cmH₂O was used throughout the protocol.

An arterial catheter was placed in the carotid artery for constant measurement of blood pressure and periodic blood sampling for analysis of arterial pH and blood gases. Arterial blood samples (~100 µl/sample) were removed at selected time periods and analyzed for arterial PCO₂, PO₂, and pH by using a blood-gas analyzer (model 1610, Instrumentation Laboratories). Before analysis, the analyzer was calibrated by using standardized gases and pH solutions.

A venous catheter was placed in the jugular vein to permit the infusion of isotonic saline and pentobarbital sodium when necessary. Anesthesia was maintained over the entire period of MV by repeating infusions of pentobarbital sodium (8–12 mg/kg body wt). Note that we continuously monitored the level of anesthesia in our animals by several methods (i.e., monitoring heart rate, blood pressure, and corneal and lid reflexes). Body temperature was maintained at ~37°C by use of a recirculating heating blanket. Additionally, heart rate and electrical activity of the heart were monitored via a lead II electrocardiogram by using needle electrodes placed subcutaneously.

Throughout MV animals received enteral nutrition from the aforementioned AIN-76 rodent diet. This planned feeding schedule was identical to the routine described for the spontaneously breathing animals. Furthermore, body fluid homeostasis was maintained via the administration of 2.0 ml·kg⁻¹·h⁻¹ intravenous electrolyte solution.

Continuing care during MV included expressing the bladder, removing airway mucus, lubricating the eyes, rotating the animal, and passively moving the limbs. This care was maintained throughout the experimental period at 2-h intervals. Finally, identical to the protocol used in the spontaneously breathing animals, repeated intramuscular injections of glycopyrrolate (0.04 mg·kg⁻¹·h⁻¹) were employed to reduce airway secretions. On completion of MV, a segment of the costal diaphragm was removed for in vitro measurement of contractile properties.

Measurement of in Vitro Diaphragmatic Contractile Properties

Description of the muscle organ bath. Details of our in vitro protocol have been described in detail elsewhere (6, 10, 17, 18) and will be briefly described here. After a surgical plane of anesthesia was reached, the entire diaphragm was removed and placed in a dissection chamber containing a Krebs-Henselit solution equilibrated with a 95% O₂-5% CO₂ gas. A muscle strip, including the tendinous attachments at the central tendon and rib cage (dimensions = 20 ± 3 mm), was dissected from the midcostal region. The strip was suspended vertically between two lightweight Plexiglas clamps with one end connected to an isometric force transducer (model PT-03, Grass Instruments, Quincy, MA) within a jacketed tissue bath. The force output was recorded via a computerized data-acquisition system (Super Scope II, GW Instruments Somerville, MA; Apple Computer Cupertino, CA). The tissue bath was filled with Krebs-Henselit saline and 12 µM d-tubocurarine to produce complete neuromuscular blockade. The saline within the bath was aerated with gas (95% O₂-5% CO₂), pH was maintained at 7.4, and the osmolality of the bath was ~290 mosmol/kgH₂O.

Determination of the optimal length-tension relationship. After a 15-min equilibration period (25°C), in vitro diaphragmatic contractile measurements were made. The muscle strip was stimulated along its entire length with platinum wire electrodes (modified 548 stimulator, Grass Instruments) by using supramaximal (~150%) stimulation voltage to determine the optimum contractile length (L₀). L₀ was determined by systematically adjusting the length of the muscle by using a micrometer while evoking single twitches. Thereafter, all contractile properties were measured isometrically at L₀.

Peak twitch and tetanic tension. After L₀ was found, the bath temperature was increased to 37°C, and, after a 30-min equilibration period, peak twitch tension was determined by using supramaximal monophasic pulses of 2 ms; peak isometric twitch tension was then determined from a series of single pulses. Maximal isometric tetanic contractions (P₀) were then produced by using a supramaximal stimulus train of 500-ms duration (160 Hz). Force was monitored by the computerized system described in Description of the muscle organ bath. Peak isometric twitch tension was measured from a series of three contractions with a 3-min recovery between measurements.

Force-frequency response. To measure the force frequency response each strip was stimulated supramaximally with 120-V pulses at 15–160 Hz. The duration of each train was 500 ms to achieve a force plateau. Contractions were separated by a 3-min recovery period.

Normalization of diaphragm force production to muscle cross sectional area. For comparative purposes, diaphragmatic (bundles of fibers) force production was normalized as specific P₀. The total muscle cross-sectional area at right angles to the long axis was calculated by the following algorithm (22)

\[
\text{Total muscle cross-sectional area (mm}^2\) = \left[\text{muscle mass}/(\text{fiber length} \times 1.056)\right]
\]

where 1.056 is the density of muscle (in g/cm³). Fiber length was expressed in centimeters measured at L₀.

Postmortem Examination

To determine whether our animals developed an infection during our experiments three separate blood samples were removed, smeared on microscope slides, and stained by using a bacteriological stain (Gram’s stain, Sigma Chemical, St. Louis, MO); each sample was then microscopically examined for the presence of bacteria. Furthermore, lungs were visually examined for abnormalities. If macroscopic abnormalities of the lung were observed during necropsy, these animals were eliminated from the study.

Statistical Analysis

Comparisons between groups for each dependent variable measured were made by a two-way ANOVA. Where significant differences existed, a Scheffe’s test was used post hoc. Furthermore, the relationship between the duration of MV and diaphragmatic specific P₀ was examined by using a Pearson product-moment correlation. Significance was established at \(P < 0.05\).

RESULTS

Systemic and Biological Response to MV

Two animals that completed the prescribed period of MV were eliminated from our analysis because postmortem analysis revealed lung barotrauma. No animals were eliminated because of infection. Of the animals included in our analysis, initial and final body weights did not differ (\(P > 0.05\)) between the experi-
Table 1. Changes in arterial Po2, Pco2, and pH at selected time periods during mechanical ventilation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 h of MV (n = 17)</th>
<th>12 h of MV (n = 17)</th>
<th>24 h of MV (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, Torr</td>
<td>94 ± 4</td>
<td>89 ± 4</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>37 ± 2</td>
<td>38 ± 2</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.44 ± 0.04</td>
<td>7.42 ± 0.04</td>
<td>7.44 ± 0.05</td>
</tr>
<tr>
<td>Systolic arterial blood pressure, mmHg</td>
<td>110 ± 6</td>
<td>103 ± 5</td>
<td>98 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, total no. of animals that were exposed to mechanical ventilation (MV) for each time period. PaO2, arterial Po2; Paco2, arterial Pco2; pH, arterial pH.

Table 2. Changes in arterial Po2, Pco2, and pH at selected time periods during 18 h of spontaneous breathing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 h of Spontaneous Breathing</th>
<th>12 h of Spontaneous Breathing</th>
<th>18 h of Spontaneous Breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, Torr</td>
<td>56 ± 4</td>
<td>63 ± 3</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>50 ± 4</td>
<td>52 ± 2</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.02</td>
<td>7.36 ± 0.02</td>
<td>7.37 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 4 animals for each time period.
prolonged MV in anesthetized animals is not due to a direct effect of pentobarbital sodium on the diaphragm. Furthermore, our results indicate that as few as 12 h of controlled MV result in decreased submaximal diaphragmatic specific force production and $P_o$. Finally, the magnitude of diaphragmatic contractile dysfunction increased progressively with time on the ventilator. Therefore, these data support the hypothesis that the onset of MV-induced diaphragm dysfunction is rapid and that this contractile impairment is exacerbated with increased time on the ventilator.

**Prolonged MV and Diaphragmatic Contractile Dysfunction**

The present experiments support previous reports (1, 15) that prolonged MV results in a significant reduction in both submaximal diaphragmatic specific force production and $P_o$. Importantly, the magnitude of diaphragmatic contractile dysfunction increased progressively with time on the ventilator. Therefore, these data support the hypothesis that the onset of MV-induced diaphragm dysfunction is rapid and that this contractile impairment is exacerbated with increased time on the ventilator.

**Table 3. Maximal isometric twitch force production in diaphragm strips obtained from mechanically ventilated and nonmechanically ventilated (control) animals**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Maximal Isometric Twitch Force, N/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>18 h of spontaneous breathing</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>24 h of spontaneous breathing</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>12 h of MV</td>
<td>4.3 ± 0.3†</td>
</tr>
<tr>
<td>18 h of MV</td>
<td>3.4 ± 0.1*</td>
</tr>
<tr>
<td>24 h of MV</td>
<td>3.8 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Different from control and both spontaneous breathing groups, $P < 0.05$. †Different from all other experimental groups; $P < 0.05$.

Although these experiments were not designed to determine the mechanism(s) responsible for the MV-induced decrease in diaphragmatic specific force production, a discussion of this issue is warranted. There are at least four possible explanations for the diaphragmatic contractile dysfunction associated with prolonged MV: 1) negative impact of anesthesia on muscle function, 2) infection, 3) arterial pH imbalance, and/or 4) intrinsic alterations in diaphragm muscle fibers. We postulate that the MV-induced diaphragmatic contractile dysfunction was not due to anesthesia, infection, or a pH/blood-gas disturbance. Arguments to support this position follow.

High levels of barbiturates have been reported to influence mammalian muscle contractile properties (25). Nonetheless, in the present experiments, the level of pentobarbital sodium required to maintain a surgical plane of anesthesia did not impair diaphragm-
mechanical function in the two groups of spontaneously breathing animals. Hence, these data indicate that the level of pentobarbital sodium anesthesia used in our experiments does not contribute to the diaphragmatic contractile dysfunction associated with prolonged MV.

Although sepsis is associated with impairment of diaphragmatic force production (2), our data indicate that our animals did not develop infections during MV. Therefore, these results indicate that sepsis was not responsible for the diaphragmatic force deficit associated with MV.

It is well known that arterial acidosis decreases muscle force production and increases fatigue in the diaphragm (4, 8). Nonetheless, our measurements of arterial pH indicate that acid-base status was well maintained during 24 h of MV (Table 1). Hence, we do not believe that the diaphragmatic contractile dysfunction associated with prolonged MV is linked to systemic acidosis. Our finding that diaphragmatic contractile properties were not impaired in our spontaneously breathing animals that experienced 18–24 h systemic acidosis supports this notion.

By elimination, we conclude that the MV-induced contractile dysfunction is due to intrinsic changes within diaphragmatic muscle fibers. Theoretically, the MV-induced diaphragmatic force deficit could stem from one or several different mechanisms. Specifically, a reduction in myofibrillar protein concentration, abnormalities of contractile or cytoskeletal proteins, and/or impaired excitation-contraction (E-C) coupling could individually or collectively contribute to the observed force deficit (5, 10). If individual myosin cross bridges produce the same force when activated, the force produced by a muscle is proportional to the number of parallel cross bridges in the strong binding state (5). It follows that a reduction in the number of cross bridges (i.e., myofibrillar protein) per cross-sectional area of muscle would result in a reduced specific force production. Therefore, it is possible that a reduction in myofibrillar protein concentration is a potential contributory factor to the MV-induced diaphragmatic specific force deficit.

A second potential mechanism that may contribute to the MV-induced diaphragmatic force deficit is an abnormality in either contractile or cytoskeletal proteins. That is, intrinsic alterations in the structure and function of key contractile or cytoskeletal proteins could impair diaphragmatic force generation. Although preliminary experiments indicate that diaphragmatic contractions do not occur during prolonged controlled MV, we cannot eliminate the possibility that an occasional, nonsynchronized inspiratory effort occurred during our experiments. This type of diaphragmatic contraction could result in muscle injury and therefore reduce diaphragmatic force production. Also, it is possible that prolonged MV is associated with elevated diaphragmatic proteolysis and increased oxidative damage to muscle proteins. Each of these factors can promote intrinsic alterations in muscle proteins and could impair force production (9, 12, 19–21).

A final potential mechanism to explain the MV-induced diaphragmatic force deficit is impairment in E-C coupling. For example, a reduction in calcium release from the sarcoplasmic reticulum is associated with reduced muscle force generation (21). Furthermore, a reduction in myofilament sensitivity to calcium is also associated with lowered force production (21). Hence, any factor that reduces the magnitude of calcium release from the sarcoplasmic reticulum and/or calcium sensitivity would negatively impact muscle force production. The data of the present study do not provide evidence for or against the possibility that E-C coupling in the diaphragm was impaired by MV. Nonetheless, our experiments reveal that the onset of MV-induced diaphragmatic contractile dysfunction occurs rapidly and is progressive during the first 24 h of MV. These observations indicate that the pathways responsible for this contractile dysfunction are activated quickly after the induction of MV. In this regard, it seems possible that the activation of oxidant production and/or proteolytic pathways in the diaphragm during prolonged MV are candidate mechanisms that could negatively impact E-C coupling and muscle force production (7, 19, 21).

In summary, we postulate that MV-induced diaphragmatic contractile dysfunction is due to intrinsic changes within the diaphragmatic muscle fibers. In theory, the MV-induced diaphragmatic force deficit could result from a reduction in myofibrillar protein concentration, abnormalities of contractile or cytoskeletal proteins, and/or impairment in calcium handling (i.e., E-C coupling) in the diaphragm. Which of these specific mechanisms is responsible for the observed force deficit is unknown and is an important area for future research.

Critique of Experimental Model

Because of the invasive nature of obtaining a biopsy from the diaphragm in human patients, animal models must be used to study the effects of MV on respiratory muscle biochemistry and in vitro contractile properties. Considerations in the choice of an animal model include both practical considerations (i.e., size of the animal and cost) and the applicability of the animal model to humans. In this regard, the rat is a relatively inexpensive model to study the effects of MV. Most importantly, human and rat diaphragms are similar in fiber-type composition, gross anatomic features, and function (16, 18). Therefore, we chose the rat model to study the effects of prolonged MV on diaphragmatic function.

Experiments that use animal models are obligated to use only the number of animals required to answer the question addressed in a particular experiment; this was the approach used in the present experiments. Our results reveal that only a small sample size in each of our experimental groups was required to observe a statistically significant difference in diaphragmatic
force production between control animals and MV animals. This is a reflection of the large between-group mean differences (i.e., control vs. MV) in muscle force production and the relatively low variability in the data. Importantly, the application of parametric statistics with a specified alpha level (i.e., $P < 0.05$) to a given data set ensures that the chances of making a type I error is the same in all experiments independent of the number of observations. Hence, the results of the present experiments can be viewed with the same level of confidence as any other experiments using an alpha of $P < 0.05$.

It is noteworthy that most adult patients receiving MV in intensive care units are maintained on some form of pressure-assist MV. Nonetheless, to investigate the effects of prolonged MV on diaphragmatic contractile function, we chose to use controlled MV for two primary reasons. First, compared with assist modes of MV, an advantage of controlled MV is that this mode is postulated to result in rapid diaphragmatic atrophy (26). This is experimentally advantageous because it permits the investigation of the adverse effects of MV on the diaphragm after relatively short periods of MV. Second, controlled MV has clinical relevance because this mode of ventilation is used in adult patients in several circumstances, including drug overdose, spinal cord injury, and surgery. Furthermore, controlled MV is commonly used in numerous pediatric situations (11).

We chose pentobarbital sodium as the general anesthetic in these experiments because of evidence that this anesthetic, when used in low doses, does not negatively impact locomotor and diaphragm contractile function. For example, work by Le Bourdelles et al. (15) reveals that the level of pentobarbital sodium required to maintain a surgical plane of anesthesia in rats over a 48-h period does not promote locomotor muscle atrophy, nor does it impair locomotor muscle maximal tetanic force production. Importantly, the present experiments with anesthetized, spontaneously breathing animals clearly indicate that the level of pentobarbital sodium required to maintain a surgical plane of anesthesia does not impair diaphragm contractile function.

**Summary and Conclusions**

These novel experiments examined the time course of MV-induced contractile dysfunction in the rat diaphragm. Our data indicate that as few as 12 h of MV result in decreased diaphragmatic specific force production and that the magnitude of this force deficit increases as a function of time on the ventilator. The mechanism(s) responsible for this MV-induced contractile dysfunction is unclear. Nonetheless, we postulate that the observed MV-induced diaphragmatic contractile dysfunction is linked to intrinsic changes within diaphragm muscle fibers. Future experiments designed to ascertain those factors that trigger these injurious processes in the diaphragm will be required to develop a clinical intervention to prevent diaphragmatic injury during prolonged MV.

This work was supported by a grant from the American Lung Association, Florida Affiliate, and by National Heart, Lung, and Blood Institute Grant R01 HL-62361 (to S. K. Powers)

**REFERENCES**


