Recovery of Costal and Crural Diaphragmatic Contractility from Partial Paralysis

E. Goldman, M.D.,* J. Road, M.D.,† A. Grassino, M.D.‡

Since the two muscles (costal and crural) that constitute the diaphragm are separate and histologically different, their individual recovery pattern from neuromuscular blockade also may be different. Therefore, we studied the recovery of force and shortening in the in vivo diaphragm from atracurium-induced neuromuscular blockade in seven pentobarbital anesthetized dogs to assess segmental differences. Transdiaphragmatic pressure (Pdi), shortening of costal and crural segments, integrated electromyogram (EMG), and tidal volume (VT) were measured during spontaneous breathing. After atracurium had reduced VT to 30% of control, breathing parameters were followed until recovered to 90% of control values. In addition, force-frequency curves generated by supramaximal tetanic stimuli of the phrenic nerve were measured. Recovery times for tidal Pdi, tidal EMG, tidal shortening, low-frequency shortening, and twitch Pdi were twice as fast as for VT (40 ± 4 min), reflecting a slower rate of recovery of accessory inspiratory muscles. High-frequency recovery was typically slower than that of VT. During tidal breathing and tetanic stimulation, costal and crural shortening recovered simultaneously. On the other hand, comparison between costal and crural by analysis of pressure–shortening relationships showed a segmental difference (crural shortened 30% more than costal at the same Pdi), which implied reduced afterload on the crural segment. However, since shortening and pressure were linearly related during paralysis and recovery, measurements of Pdi alone can accurately reflect changes in contractile mass when heterogeneity and afterload are controlled. (Key words: Muscles, diaphragm: contractility. Measurement techniques: sonomicrometry; electromyography. Neuromuscular relaxants: atracurium.)

DIAPHRAGMATIC CONTRACTILITY has been measured in humans1–4 and animals5 during recovery from neuromuscular blockade via transdiaphragmatic pressure (Pdi) swing elicited by single pulses of electrical stimulation applied to the phrenic nerve (single-twitch Pdi). Recovery of skeletal muscle function from neuromuscular blockade follows a predictable sequence: recovery of the diaphragm always precedes that of the adductor pollicis1,4,6 or other respiratory muscles. However, the single-twitch Pdi is affected by several variables not dependent on diaphragm contractility alone, and therefore doubt is cast on its validity as an expression of diaphragmatic properties. For example, lung volume,7 initial length of the diaphragm,7 state of recovery from fatigue,8 elastance of the chest wall,9 and posttetanic potentiation or facilitation10 all are factors affecting the twitch Pdi. A more precise evaluation of diaphragm function may be obtained if direct measurement of diaphragmatic length and Pdi swings are done simultaneously during a twitch or tetanic stimulation.11,9

There are no reports in the literature on the use of both methods to evaluate recovery from neuromuscular blockade in the in vivo diaphragm. Furthermore, the diaphragm is composed of two separate muscles (costal and crural12,13), and since these differ in fiber composition, neural innervation, and mechanical actions, their individual recovery pattern from neuromuscular blockade may be different. In this work we measured the evolution of various breathing cycle parameters, force–frequency curves, and the mechanical characteristics in both parts of the dog's diaphragm in vivo during recovery from partial paralysis induced by atracurium.

Materials and Methods

Experiments were approved by the Animal Care Committee of McGill University. Six mongrel dogs (18–25 kg body weight) were studied. Anesthesia was induced with 30 mg/kg intravenous (iv) pentobarbital and supplemented with intermittent doses of 2–3 mg/kg to keep a level of anesthesia that just maintained the corneal reflex. The trachea was intubated with a number-9 cuffed endotracheal tube, and each dog was placed in the supine position for the duration of the experiment. An arterial catheter was inserted into the femoral artery to record blood pressure. A peripheral venous catheter allowed administration of fluid and anesthetic. Temperature was monitored with a rectal probe and kept constant with an electrical heating blanket. Temperature ranged between 36.2° and 38° C.

Two pairs of piezoelectric transducers (crystals), 2 mm in diameter, were then placed between the fibers of the left costal (at the level of the midaxillary line) and the left crural diaphragm (medial to the inferior phrenic artery) via a midline laparotomy. Each pair was placed 10–15 mm apart along the same fiber bundle and secured with sutures within the diaphragm. The crystals were con-
nected to an ultrasonic device (sonomicrometer model 120, Triton Technology, San Diego, CA) via fine insulated wires. This method allows accurate measurement of diaphragmatic length, as previously reported. The sonomicrometer direct-current output was proportional to the diaphragm length and was calibrated in millimeters. Diaphragm length at end-expiration (i.e., at functional residual capacity [FRC]) was termed resting length (LFRG). Diaphragmatic changes in shortening during tidal breathing or tetanic stimulation were expressed as percentage change from the initial LFRG (Δ%LFRG).

Bipolar silver-coated hook electrodes were inserted in fibers parallel to both pairs of crystals for measurement of the diaphragm electromyogram (costal and crural EMG). The signal was amplified (EMG 05A02, DISA) and filtered to include the range of 50–1,000 Hz. The output was integrated through a resistance capacitance filter (time constant 100 ms). EMG activity was measured as the peak integrated value. One 5-cm latex balloon was inserted in the mid-esophagus, and a second was placed in the abdomen under the central tendon for the measurement of esophageal and abdominal pressure. The esophageal balloon was filled with 0.5 ml air and the abdominal balloon with 1.0 ml air. The balloons were connected via 50-cm PE-200 tubing to a differential pressure transducer (model BC267, Hewlett-Packard, range 0–400 mmHg). Pdi was measured as the difference between abdominal pressure and esophageal pressure. After positioning of the electrodes and balloons, the abdominal wall was closed.

Phrenic nerve roots (C5–C6) were isolated bilaterally by an anterior midline incision in the neck. A stainless steel stimulating electrode was placed on each root and insulated by immersion in mineral oil. An additional electrode on the right C5 root (cut central end) recorded phrenic nerve output (electroneurogram [ENG]). The signal was filtered (range 50–5,000 Hz), and the peak integrated value was measured.

The dogs breathed spontaneously from a bag reservoir (fractional inspired oxygen concentration [F-I02] 0.4, balance nitrogen) through a two-way valve (H. Rudolph Inc., Kansas City, MO). A Fleisch number-1 pneumotachograph was attached between the two-way valve and the distal end of the endotracheal tube. The electrical signal was integrated to give tidal volume (VT). All measurements were recorded on a strip-chart recorder (model 7758A, Hewlett-Packard). End-tidal carbon dioxide (partial pressure, in millimeters mercury) was measured with a mass spectrometer.

Tidal Breathing Protocol

While the anesthetized dogs were breathing spontaneously, costal and crural tidal shortening of the dia-

phragm (Δ%LFRG), peak integrated EMG, ENG, VT, and Pdi were measured on ten consecutive breaths, and the results were averaged and recorded as control. Atracurium was then given iv (0.2 mg/kg), and the same variables were measured at 30- to 60-s intervals. Thereafter, the last set of measurements was obtained when VT was reduced to 30% of control (V_T90). If apnea was induced, the dog's lungs were mechanically ventilated. The effect of the atracurium-induced neuromuscular blockade was allowed to reverse spontaneously. When VT spontaneously resumed, all variables were measured every 2 min until VT reached 90% of control values (VT90). Recovery of EMG, ENG, diaphragm shortening, and Pdi was considered completed when each variable first reached 90% of its own control value, which occurred in less than the VT90 recovery time.

FORCE–FREQUENCY CURVES: Protocol

The dog's abdomen then was casted firmly up to the lower rib cage. The objective was to stabilize diaphragm movement during measurement of force–frequency curves (the cast was on only for the stimulation period). Force was measured as Pdi. Diaphragmatic shortening was expressed as a percent change from the length at the end of spontaneous VT (Δ%LFRG, costal and crural). Only maximum change was recorded (without including the fade). Supramaximal voltage for phrenic nerve stimulation was set at 20% above that which produced a maximum change in Pdi. The stimulus was applied to the C5–C6 phrenic nerve roots in the neck at 5, 10, 20, 50, and 100 Hz during 1-2 s for each frequency. The interval between each stimulation was about 5 s. Since posttetanic potentiation and facilitation (see discussion) may affect the force elicited during successive stimulating frequencies, the order in which the frequencies were applied was randomized. Successive force–frequency curves with frequencies applied in the reverse order showed no potentiation or facilitation effect. Force–frequency curves following neuromuscular blockade (atracurium 0.2 mg/kg iv) were measured periodically with the same protocol described above for spontaneous recovery. Since recovery of pressure and shortening at high frequencies were in general below 90% of control at VT90, data were measured as the peak value at VT90.

Supramaximal train of four stimuli (0.2 ms at 2 Hz) were applied simultaneously to the C5–C6 phrenic nerve roots. Twitch Pdi was measured as the peak Pdi swing. Twitch diaphragmatic shortening was also recorded for the costal and crural diaphragms. The ratio of first twitch to control (T1/T0) was recorded. Twitch stimulation to the diaphragm was applied 1–5 min apart from the force–frequency curves in order to minimize posttetanic potentiation and facilitation effects.
Data are presented as mean ± standard error of the mean. The unpaired Student's t test was used to detect differences in recovery time (table 1). Differences when V T30 values were compared to control and recovery (for each variable) were assessed using analysis of variance with the Student-Newman-Keuls multiple-range test for analysis of individual means. A P value < 0.05 was considered statistically significant. Force–frequency curves (Pdi and crural and costal shortening vs. frequency of stimulation) were analyzed using a general linear model (SAS) and compared by an analysis of variance.

Results

Tidal Breathing Parameters

Table 1 shows each variable measured during control, V T30, and recovery of each parameter to 90% of its control value. Both electrical (EMG for costal and crural) and mechanical (Pdi and tidal shortening in Δ%LFR) for costal and crural variables were significantly reduced with respect to control values (P < .01). End-tidal carbon dioxide partial pressure was 44 ± 9 mmHg. The control resting length (LFR) of costal (11 ± 1 mm) and crural (10 ± 1 mm) diaphragm (at the end of expiration) remained unchanged at V T30 and at recovery. The phrenic nerve EMG was significantly increased with respect to control values, indicating an increased respiratory motor neuron output, whereas the diaphragm response was partially blocked by atracurium. Tidal integrated EMG, shortening of costal and crural segments, and Pdi recovered earlier (13–19 min) than did VT (40 ± 4 min, P < .01). Recovery of costal and crural shortening was similar (table 1).

Twitch Pdi

At V T30, twitch Pdi expressed as percent of control (T1/T0) was 57 ± 7%. Twitch Pdi (T1/T0) recovered to 93 ± 3% in 25 ± 3 min, which was not significantly different from tidal EMG, shortening, and Pdi recovery times. At V T30 and V T90, twitch stimulation generated pressure (twitch Pdi) and shortening (costal and crural), neither of which was significantly different from those obtained during 5-Hz stimulation.

Force–Frequency Curves

The response of the diaphragm to different frequencies of stimulation during control, V T30, and recovery (V T90) is shown in figure 1. Shortening and Pdi were expressed as a percent of their maximum at 100 Hz. All variables were similarly decreased by atracurium and did not recover (except below 10 Hz) at the time of V T90 (44 ± 3 min). They did recover eventually, 60 minutes after atracurium. Curves at V T30 were significantly...
Nonsustained response (fade) was always present during recovery in the pressure tracings (fig. 2), starting as low as 20 Hz and also at 50 and 100 Hz, probably because of the fade of other muscles, which contribute passively to the Pdi. In contrast, both costal and crural shortening showed no fade at the same time and same level of stimulation.

Resting length during force–frequency curves remained unchanged from control (costal 11 ± 1 mm and crural 10 ± 1 mm) during V7 30 and recovery (maximum change for costal was 3% and for crural 3.7% of resting length).

**Pressure–Shortening Relationships**

To describe the mechanical characteristics of the chest wall (elastance) and diaphragmatic contractility during neuromuscular blockade we related initial length, shortening, and pressure generation. Initial length did not change. The Pdi–shortening (Pdi/Δ%LFRC) relationships of the diaphragm obtained at all levels of tetanic stimulation during control, V7 30, and V7 90 were linear (fig. 3). Slopes for costal (2.65) and crural (1.70) segments were significantly different (P < 0.01). At a Pdi of 60 cmH2O, the costal segment shortened about 30% less than the crural.

**Discussion**

The principal findings of this study are as follows. First, the finding of a linear relationship between shortening and pressure in both the costal and crural diaphragms supports the concept that neuromuscular blockade did not alter the elastance of the chest wall and that diaphragmatic contractility can be reliably estimated by Pdi. However, the afterload for the costal was shown to be greater than for the crural diaphragm. Second, following partial paralysis of the diaphragm, costal and crural tidal shortening recovered simultaneously. Shortening during maximal stimulation also recovered simultaneously in the costal and crural diaphragms. Third, resting length of the diaphragm was not affected by neuromuscular blockade.

Finally, recovery time for tidal Pdi, integrated EMG, and tidal shortening (costal and crural) was comparable to twitch Pdi recovery time. Tidal volume took twice as long to recover as did tidal shortening and twitch Pdi. Recovery of diaphragmatic contractility in response to high-frequency stimulation was delayed beyond the recovery of V7.
covery have not been reported before in the literature. Upon stimulation from its resting length, the diaphragm shortens and generates a Pdi simultaneously. The slope of such contraction may vary, as a function of the afterload produced by the rib cage and the abdomen (elastance). The extent to which the diaphragm shortens is a function of the level of stimulation and the mass of the contracting muscle given a constant preload (initial length) and afterload. It is remarkable that during control and over various levels of neuromuscular blockade (recovery), the relationship remained constant. This is shown as the linear relationship for either the costal or crural diaphragms under all frequencies of stimulation and at all levels of neuromuscular blockade (fig. 3). This relationship suggests that loss of contractile muscle mass (due to partial paralysis) simply leaves the remaining muscle with the same contractile properties.

Initial length did not change during neuromuscular blockade, and thus the preload was held constant. Also, there was no change in the Pdi—shortening relationship, and hence afterload must have remained the same as well. Therefore, Pdi measurements during neuromuscular blockade may be reliable estimates of diaphragmatic contractility when these conditions are satisfied. Tonic activity in the inspiratory or expiratory muscles, which may be present in this preparation, when abolished by paralysis did not have a significant effect on the diaphragmatic initial length or Pdi—shortening relationship. End-tidal carbon dioxide partial pressure at Vt 30 was considered to have no significant effect on contractility.

The pressure—shortening analysis also shows that the crural segment shortens more than does the costal, suggesting that diaphragmatic contractility during control and partial paralysis is not homogeneous and that the seg-

**Fig. 2.** Continuous recording of a typical force—frequency stimulation at 100, 50, 20, 10, and 5 Hz. Changes in transdiaphragmatic pressure (Pdi), costal (Δ%LFRC_{cos}) and crural (Δ%LFRC_{cru}) shortening (top, middle, and bottom, respectively) are shown. Measurements were obtained 80 min after atracurium. Values on top of each deflexion are expressed as a percent of control indicating partial recovery at high frequencies. Crural shortening is significantly larger than costal shortening.
ments are interdependent. If the two segments were of approximately similar initial length in relation to optimal length, they should shorten the same amount given a constant afterload. Since the costal diaphragm shortens less than does the crural at a given Pdi (fig. 3), the costal diaphragm must have been contracting against a larger afterload, since both muscles were contracting from a length close to the optimal length. In the current study, the abdominal cast stabilized the diaphragm by partially restriction of its movement, which is responsible for part of the afterload. Complete freedom of diaphragmatic movement would allow an increase in shortening and hence would reduce the pressure–shortening slope. In addition, the relaxation configuration of the rib cage and abdomen does not change during submaximal paralysis, but the extent to which it can be distorted has not been tested. Further investigation is necessary to define the potential change of afterload by neuromuscular blockade when diaphragmatic contraction is completely unrestricted.

Recovery of Tidal Breathing

Maximum Pdi and inspiratory capacity have been shown to decrease during neuromuscular blockade. Rib cage expansion is also known to decrease due to loss of inspiratory activity of the intercostal muscles. At a high level of neuromuscular blockade (V_{T,30}), our data shows that tidal shortening and EMG of both parts of the diaphragm were quantitatively less affected than V_{T}. This may partially be explained by deactivation of the rib cage muscles, as was suggested by the marked reduction in intercostal EMG (about 25% of control) as compared to slight changes in diaphragmatic EMG during partial paralysis. This may indicate that when the diaphragmatic EMG decreases to less than 50% of control, intercostal muscle activation may be considerably lower. Unfortunately, we did not measure intercostal EMG activity to confirm this observation. The two diaphragms, costal and crural, recovered simultaneously from neuromuscular blockade. The costal diaphragm has more fast twitch fibers than does the crural diaphragm. Also, the two muscles have separate innervation, and the crural diaphragm has more neural afferent nerves than does the costal. Although the exact mechanism leading to differential responses between muscle groups to neuromuscular blockade is unknown, we wondered whether those differences may influence the recovery response of these two muscles. We found no difference. The diaphragm, therefore, recovers from neuromuscular blockade as a single unit.

Resting Length

This study is the first direct demonstration by sonomicroscopy that resting length of the diaphragm is not affected by neuromuscular blockade. This confirms previous assumptions and indirect measurements that end-expiratory configuration was unchanged by partial paralysis.

Twitch and Tetanic Pdi

The twitch Pdi recovery time of 25 min following atracurium is in agreement with previously reported data in studies of humans with vecuronium and atracurium. However, no other comparison could be derived from previous studies, since tetanic stimulation and tidal shortening were not measured, and resting length was assumed constant via a pneumograph. The recovery time of twitch Pdi is comparable with the recovery time of Pdi and shortening elicited by 5- and 10-Hz stimulation. This in turn was comparable to recovery of tidal diaphragmatic shortening, which during spontaneous breathing is activated at between 10 and 15 Hz. On the other side, recovery of V_{T} was over 40 min and recovery of Pdi and shortening at 20–100 Hz stimulation was slower (about 60 min). Thus, evaluation of recovery from neuromuscular blockade by measuring the twitch Pdi response typically underestimates the magnitude of the neuromuscular blockade to tetanic contractions. Delayed recovery of diaphragmatic tetanic response (fig. 1) is similar to that described in peripheral muscle.

Posttetanic potentiation of the twitch induced by previous high-frequency tetanus has been described in the nonparalyzed muscle and in the diaphragm by Smith et al. In their study, tetanic stimulation 1 s long (20–100 Hz) was followed by single twitches. A progressive augmentation of twitch Pdi and diaphragmatic shortening (up to 35% in the last twitch) was observed with the pre-twitch increase in frequency of stimulation. In our study, twitch response was measured 1–5 min after the preceding tetanic stimulation, which should be long enough to extinguish the potentiation or facilitation effect of tetanic contraction.

In conclusion, we report the global and segmental characteristics of the in vivo diaphragm during partial neuromuscular blockade and its recovery. Costal and crural recovery were similar both during tidal breathing and tetanic stimulation. However, analysis of the force–shortening relationships shows that the diaphragmatic afterload affects segmental contractility. Since shortening and pressure were linearly related during paralysis and recovery, changes in Pdi may accurately reflect changes in contractile mass when inhomogeneity and afterload are controlled.

References

DIAPHRAGMATIC CONTRACTILITY UNDER ATRACURIUM