J Anesth (2008) 22:236–241
DOI 10.1007/s00540-008-0613-7

Journal of
Anesthesia

Different recovery of the train-of-four ratio from rocuronium-induced neuromuscular blockade in the diaphragm and the tibialis anterior muscle in rat

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Abstract
Purpose. To clarify differences between the diaphragm and the limb muscles in terms of the effects of neuromuscular blockers concerning train-of-four (TOF) ratios, we compared the recovery of twitch tensions and TOF ratios in the diaphragm and in the tibialis anterior muscle in rats in vivo.
Methods. We conducted a dose-response study in 16 rats and a recovery study in 8 rats. In the recovery study, we made phrenic nerve-diaphragm and sciatic nerve-tibialis anterior preparations simultaneously in each of 8 rats that were anesthetized intraperitoneally with pentobarbitone (30 mg·kg^{-1}) and urethane (500 mg·kg^{-1}). After supramaximal stimuli were applied simultaneously in a TOF pattern to both the phrenic and sciatic nerves, rocuronium was injected intravenously, at 10 mg·kg^{-1}. In the diaphragm and the tibialis anterior muscle, we monitored the first-twitch response to TOF stimuli (T1) and also the TOF ratios. The following variables were determined for each muscle: (1) the times at which T1 recovered to 25%, 50%, and 75% of control T1, and the times at which the TOF ratio recovered to 25%, 50%, and 75%; and (2) the values of the TOF ratio at 25%, 50%, and 75% recovery of T1.
Results. At 25%, 50%, and 75% recovery of T1 in the diaphragm, TOF ratios were 8.9 ± 5.0 %, 26.7 ± 7.7 %, and 55.9 ± 5.4%, respectively, while in the tibialis anterior, the TOF ratios were 18.0 ± 5.9%, 32.5 ± 7.4%, and 54.4 ± 7.5%, respectively (diaphragm vs tibialis anterior; $P < 0.01$ for comparisons at both 25% and 50% recovery of T1).
Conclusion. Our method of simultaneous in vivo evaluation of TOF ratios in both the diaphragm and the tibialis anterior confirmed significant differences between the two muscles in relationships between first-twitch tension and the TOF ratio.

Key words Diaphragm · Rocuronium · Tibialis anterior muscle · Train-of-four ratio

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Received: May 14, 2007 / Accepted: February 3, 2008

Introduction

The estimation of the degree of neuromuscular blockade by measuring single-twitch tension requires a control value for twitch tension, which is obtained before any neuromuscular blocking drug is administered. The monitoring of train-of-four (TOF) ratios assumes a degree of neuromuscular blockade even when the monitoring is initiated after administration of the neuromuscular blocker. Accordingly, the TOF ratio is often used in daily clinical anesthesiological practice to determine the degree of neuromuscular blockade, and it is generally measured in a limb muscle. The degree of neuromuscular blockade in other muscles is guessed based the TOF ratio values in the limb muscle. In order to make this guess as accurate as possible, the relationship of recovery from neuromuscular blockade, in terms of twitch tension and TOF ratio, in the limb muscle and the other muscles is an important consideration, because nondepolarizing neuromuscular blocking drugs show different potencies in individual muscles. The diaphragm has been reported to be less sensitive than limb muscles to nondepolarizing neuromuscular blocking drugs, and to recover more rapidly from the blockade [1–5]. However, the relationships between twitch tension and the TOF ratio in the diaphragm and limb muscles have not been studied in detail.

In the present in vivo study of differences in recovery from rocuronium-induced neuromuscular blockade between the diaphragm and tibialis anterior muscle, we simultaneously measured twitch tensions in relation to TOF stimuli during recovery from neuromuscular blockade in the diaphragm and the tibialis anterior in rats. In addition, we carried out a preliminary experiment to decide the dosage of rocuronium and also to verify the reliability of the actual experiment. In the preliminary experiment, sensitivity to rocuronium in the diaphragm and the tibialis anterior was investigated

with the nerve-muscle preparations used in the actual experiment.

Materials and methods

We conducted this study in 24 male Sprague-Dawley rats (weight, 330 to 430 g) after obtaining approval from our institutional Animal Care and Use Committee. We used 16 rats in experiment 1 and 8 rats in experiment 2. The rats were housed in mesh cages in a room maintained at 25°C with 12-h:12-h light-dark cycles, and they were provided with standard rodent chow and water ad libitum.

On the day of the experiment, the animals were anesthetized intraperitoneally with pentobarbitone (30 $mg \cdot kg^{-1}$) and urethane (500 $mg \cdot kg^{-1}$), followed by another 15 $mg \cdot kg^{-1}$ and 250 $mg \cdot kg^{-1}$, respectively, if necessary. Ringer's lactate solution was infused at 10 $ml \cdot h^{-1}$. After tracheotomy, the lungs were ventilated with 100% O_2, using 3 cmH_2O positive end-expiratory pressure to maintain Pa_{CO_2} at approximately 40 mmHg, according to arterial blood gas analysis, while pH was maintained between 7.35 and 7.45. The external jugular vein was catheterized for drug administration, while the internal carotid artery was catheterized to monitor arterial blood pressure. Rectal temperature was maintained between 36°C and 37°C with heating pads and lamps.

Dose-response study (experiment 1; preliminary experiment; n = *16)*

*Phrenic nerve-diaphragm preparation (*n = *8)*

We made in vivo phrenic nerve-diaphragm preparations in eight rats, performing sternotomy and bilateral thoracotomy, with careful attention to hemostasis. Both phrenic nerves were identified, carefully separated from other thoracic tissues, and ligated. Bipolar platinum electrodes were attached to the peripheral portion of the nerves. The central tendon of the diaphragm was connected to a force transducer (TB-611; Nihon Kohden, Tokyo, Japan) to measure isometric contraction of the muscle. The ribs were fixed in position with a metal frame to secure the diaphragm. Loads applied to transducers were set at 20 g for the diaphragm. This level of load was chosen in a preliminary study.

*Sciatic nerve-tibialis anterior muscle preparation (*n = *8)*

To make in vivo sciatic nerve-tibialis anterior muscle preparations in eight rats, the left sciatic nerve was dissected from the gluteal space and ligated. Bipolar platinum electrodes were attached to the peripheral portion of the nerve. The distal tendon of the left tibialis anterior was isolated from the surrounding tissues and attached to the force transducer. The knee and foot were fixed in position. Loads applied to transducers were set at 30 g for the tibialis anterior muscle. This level of load was chosen in a preliminary study.

Measurement

In the phrenic nerve-diaphragm preparation, single supramaximal stimuli, 0.2 ms in duration (SEN-7103 stimulator; Nihon Kohden), were applied every 10 s to the phrenic nerves via the bipolar platinum electrodes. In the sciatic nerve-tibialis anterior muscle preparation, single supramaximal stimuli were applied in a similar manner to the sciatic nerves. Twitch tensions were recorded with a polygraph (WT-625G; Nihon Kohden). After a steady state had been maintained for 30 min or more, rocuronium was injected intravenously, at doses ranging from 0.7 to 1.2 $mg \cdot kg^{-1}$, in the phrenic nerve-diaphragm preparation, and at doses ranging from 0.40 to 0.51 $mg \cdot kg^{-1}$ in the sciatic nerve-tibialis anterior muscle preparation. These doses were chosen according to the effective doses for 50% blockade (ED50) determined in our previous experiments [6] and in a preliminary study. We applied linear regression to a dose-response curve relating the logarithm of the dose (on the abscissa) to the probit transformation of the percent maximal block (on the ordinate). Effective doses for 50% and 90% blockade (ED 50 and ED90) in each muscle were derived from the dose-response curve of each muscle, and the slopes of these curves were estimated for each preparation. The ED ratio between the two muscles (ED of diaphragm / ED of tibialis anterior) was also calculated.

Recovery study (experiment 2; n = *8)*

We made phrenic nerve-diaphragm and the sciatic nerve-tibialis anterior preparations simultaneously in each of eight rats (Fig. 1). Supramaximal stimuli, 0.2 ms in duration (SEN-7103 stimulator; Nihon Kohden), were applied simultaneously at 2 Hz for 1.5 s (TOF pattern) every 15 s to both the phrenic and sciatic nerves via bipolar platinum electrodes. After a steady state had persisted for 30 min or more, rocuronium was injected intravenously, at 10 $mg \cdot kg^{-1}$; this represented ten times the ED50 for the diaphragm. This dose was selected to block both muscles completely. In the diaphragm and the tibialis anterior muscle, we monitored the first-twitch response to TOF stimuli (T1) and also the TOF ratios. T1 and the TOF ratio were calculated as follows:

$T1\ (\%) = (T1/Tc) \times 100$ and
$TOF\ ratio\ (\%) = (T4/T1) \times 100$, where

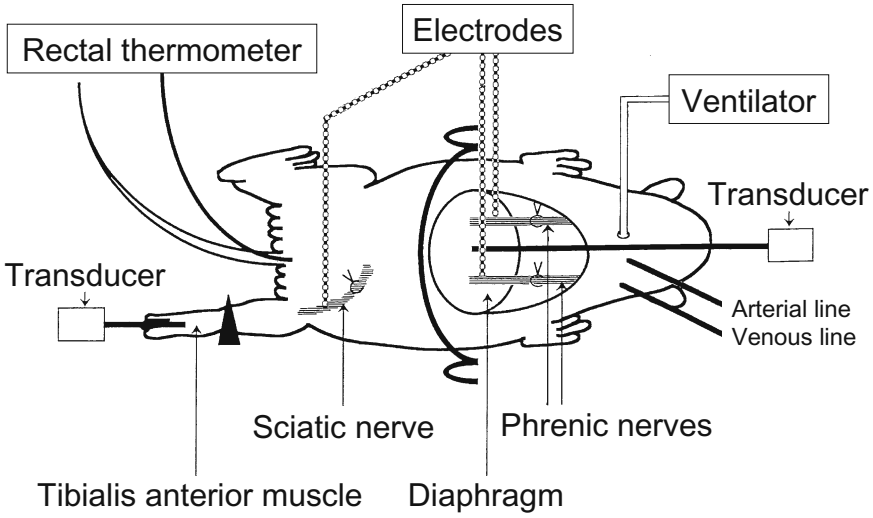

Fig. 1. Diagram of experiment 2

Tc represents the control-twitch tension before the injection of rocuronium and T4 represents the tension produced by the last-twitch response to the TOF stimuli.

The following variables were determined for each muscle: (1) the times at which T1 recovered to 25%, 50%, and 75% (recovery times at 25%, 50%, and 75% of control T1), and the times at which the TOF ratio recovered to 25%, 50%, and 75% (recovery times at 25%, 50%, and 75% of TOF ratio); (2) the recovery index, defined as the time required for recovery of T1 (%) from 25% to 75%, and as the time for recovery of the TOF ratio from 25% to 75%; and (3) the values of the TOF ratio at 25%, 50%, and 75% recovery of T1. When T1 and the TOF ratio were not measured at exactly the 25%, 50%, and 75% points, the nearest points were selected.

Statistical analysis

Paired *t* analysis was used for appropriate comparisons. Simple regression analysis was used to estimate the relationship of the recovery index between T1 and the TOF ratio in each muscle. Values for results are expressed as means (followed by the 95% confidence interval) and as means ± SD for experiments 1 and 2, respectively. Differences were considered significant when *P* was below 0.05.

Results

Intravenous administration of rocuronium had little effect on heart rate and blood pressure, and vital signs were stable throughout the experiments in all 24 rats. In experiment 1, twitch tensions in the diaphragm in 8 rats and in the tibialis anterior muscle in 8 rats recovered to 90% or more of control tensions. In experiment 2, T1 in the diaphragm and the tibialis anterior muscle in 8 rats recovered to 90% or more of control values.

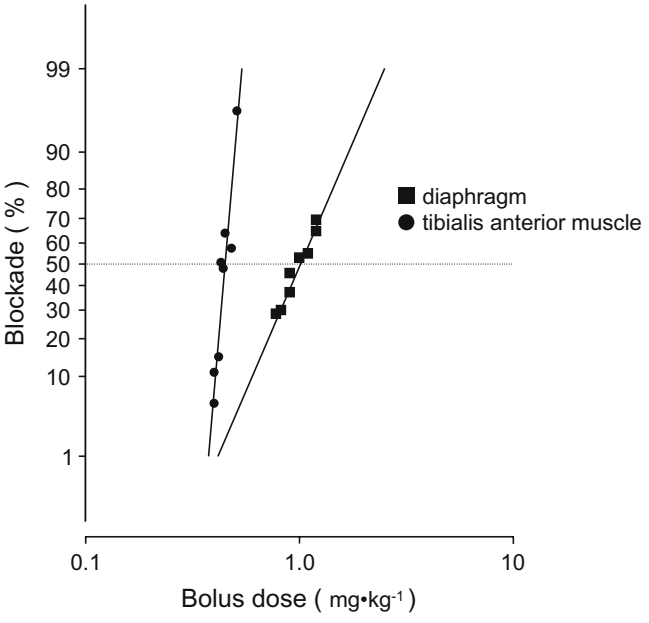

Fig. 2. Single bolus dose-response curves for muscles, showing percent blockade (probit scale) vs dose of infusion (log scale)

Sensitivity to rocuronium in each muscle

Dose-response curves for the diaphragm and tibialis anterior muscle are presented in Fig. 2, while the ED50 and ED90 values in these two muscles, and the ED ratios between the muscles are shown in Table 1. Because the 95% confidence limit for ED90 was broader than that for ED50, the ED ratio at ED50 was more

suitable as an index of the difference in sensitivity to rocuronium between the two muscles. The ED of rocuronium for the diaphragm was about twice that for the tibialis anterior muscle.

Recovery times for T1 and TOF ratio

Recovery times for T1 and the TOF ratio in the two muscles are summarized in Table 2, while the patterns of recovery of T1 and the TOF ratio for the two muscles are shown in Fig. 3. The actual values for the recovery of T1 and the TOF ratio at each data point (25%, 50%, and 75%) were considered to be acceptably close to 25%, 50%, and 75%, respectively, to permit comparison between the two muscles (no significance for any comparison). When recovery of T1 reached 25%, 50%, and 75%, each recovery time was significantly shorter in the diaphragm than in the tibialis anterior muscle ($P < 0.001$ for all comparisons). Similarly, when the TOF ratio reached 25%, 50%, and 75%, each recovery time was significantly shorter in the diaphragm than in the tibialis anterior ($P < 0.001$ for all comparisons).

Table 1. ED50 and ED90 in two muscles, with ED ratios

	ED 50 ($mg \cdot kg^{-1}$)	ED 90 ($mg \cdot kg^{-1}$)
Diaphragm	1.00 (0.96–1.05)	1.72 (0.82–2.94)
Tibialis anterior	0.45 (0.42–0.48)	0.50 (0.31–0.81)
ED ratio	2.22	3.44

Values are expressed as means (95% confidence interval)
ED, Effective dose; ED ratio, ED of diaphragm / ED of tibialis anterior

Relationship between T1 and TOF ratio

Relationships between T1 and the TOF ratio during recovery from neuromuscular blockade in the diaphragm and the tibialis anterior muscle are summarized in Table 3. TOF ratios were significantly less in the diaphragm than in the tibialis anterior muscle at 25% and at 50% recovery of T1 ($P < 0.01$ for both comparisons), while TOF ratios at 75% recovery of T1 in the two

Table 2. Recovery of T1 and of train-of-four (TOF) ratio in two muscles

	Diaphragm	Tibialis anterior	*P*
Recovery time of T1 (s)			
25% Recovery of T1	471 ± 76	872 ± 153	<0.001
50% Recovery of T1	585 ± 102	979 ± 165	<0.001
75% Recovery of T1	733 ± 110	1129 ± 212	<0.001
Recovery index of T1 (s)	263 ± 51	257 ± 66	NS
Recovery time of TOF ratio (s)			
25% TOF ratio	572 ± 82	939 ± 203	<0.001
50% TOF ratio	694 ± 104	1100 ± 206	<0.001
75% TOF ratio	863 ± 132	1240 ± 254	<0.001
Recovery index of TOF ratio (s)	292 ± 81	298 ± 73	NS

Values are expressed as means ± SD
Recovery index of T1, Time required for recovery of T1 from 25% to 75% of the control T1; recovery index of TOF ratio, time required for recovery of the TOF ratio from 25% to 75%

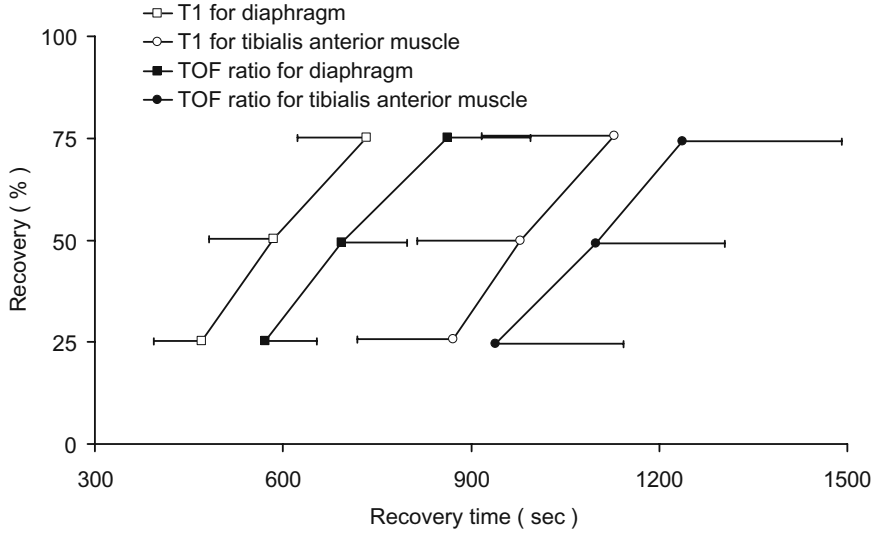

Fig. 3. Patterns of recovery of T1 and train-of-four (*TOF*) ratio for the two muscles, showing recovery time (s) vs percent recovery (%). Values are expressed as means ± SD. *T1*, First-twitch response to TOF stimuli

Table 3. Relationship between first-twitch recovery and train-of-four (TOF) ratio

	Diaphragm	Tibialis anterior	*P*
TOF ratio (%)			
25% Recovery of T1	8.9 ± 5.0	18.0 ± 5.9	<0.01
50% Recovery of T1	26.7 ± 7.7	32.5 ± 7.4	<0.01
75% Recovery of T1	55.9 ± 5.4	54.4 ± 7.5	NS

Values are expressed as means ± SD

muscles were not significantly different. These results suggest that the relationships between T1 and the TOF ratio differed between the two muscles at each level of T1.

Comparison of recovery indices for T1 and for the TOF ratio between muscles

The recovery indices for T1 and the TOF ratio did not differ significantly between the two muscles (Table 2). The relationship between the recovery indices for T1 and for the TOF ratio in the diaphragm and tibialis anterior muscle in eight rats is shown in Fig. 4. Simple regression analysis indicated a strong significant relationship between the two recovery indices in each muscle: faster T1 recovery was associated with faster TOF ratio recovery.

Discussion

When we previously studied the ED50 of rocuronium for the diaphragm and the tibialis anterior muscle by cumulative-infusion drug administration in an animal model [6], the ED ratio at ED50 was 2.30. Thus, the ED ratios at ED50 according to the two methods were similar, while Cantineau et al. [4] reported that the ED ratio, at ED50, between the diaphragm and the adductor pollicis muscle in human subjects was 1.86. Because the ED ratio at ED50 shown in our preliminary experiment was similar to the values in the previous studies, the two nerve-muscle preparations used in the preliminary experiment were thought to be suitable for evaluation of the recovery from rocuronium-induced neuromuscular blockade.

Our present data in experiment 2 showed the characteristics of the recovery of T1 and the TOF ratio in the diaphragm and the tibialis anterior.

First, in the early stage of T1 recovery, the TOF ratios at each level of T1 were significantly greater in the tibialis anterior muscle than in the diaphragm, with the difference becoming smaller as T1 recovered. On the other hand, Abdulatif et al. [7] reported that, in humans,

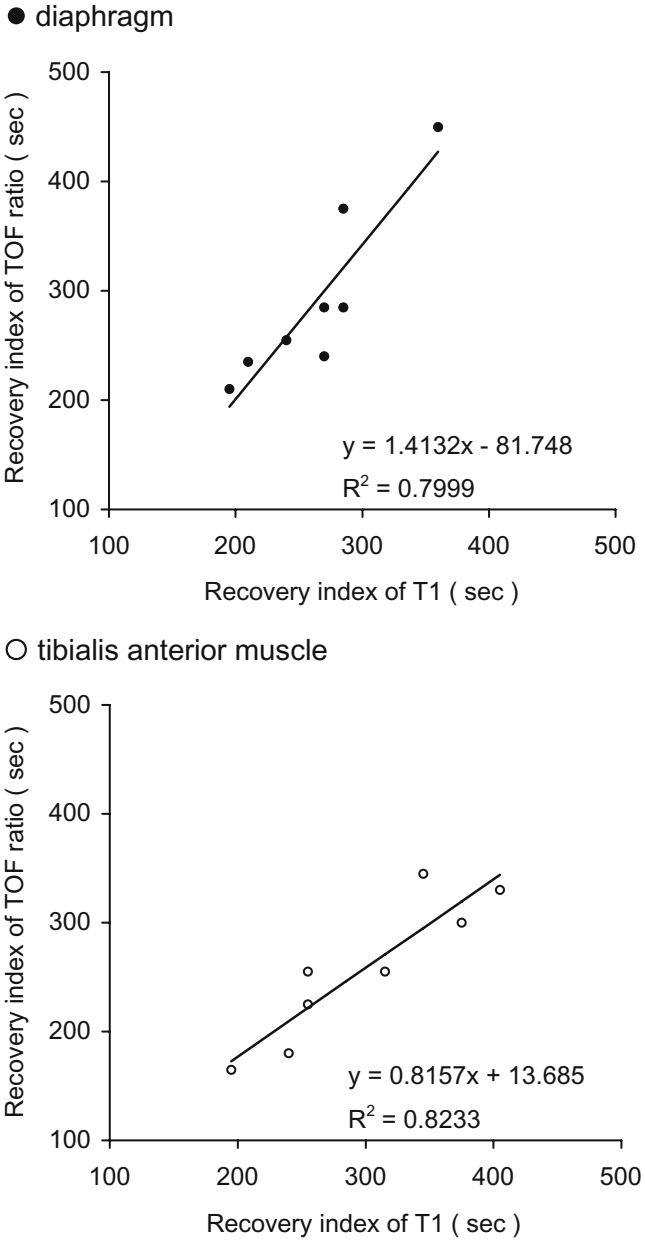

Fig. 4. Relationships between recovery indices of T1 and train-of-four (*TOF*) ratio in the two muscles in eight rats, by simple regression analysis. *T1*, First-twitch response to TOF stimuli

TOF ratios at each level of T1 were significantly greater in the orbicularis oculi than in the adductor pollicis muscle throughout the course of recovery from mivacurium-induced neuromuscular blockade.

It is thought that acetylcholine receptors (AchRs) are present on both the neuromuscular prejunction (motor nerve terminal) and postjunction (muscle) [8–11], and that the binding of nondepolarizing neuromuscular blocking drugs to prejunctional AchRs causes TOF fade [12–14]. The correlation between T1 and the TOF ratio may reflect the relative effects of these drugs on these different receptors. However, the matter remains controversial. Bartkowski et al. [15] constructed a computer model of a muscle, including multiple compartments with different blood supplies, to study relationships between T1 and the TOF ratio after the administration of a neuromuscular blocker; changes in the distribution of the drug by blood flow and diffusion in the muscle was also one of the causes of TOF fade. Nonuniformity of blood flow may be different in different muscles. Accordingly, relationships between the recoveries of T1 and the TOF ratio may be different among the diaphragm, the orbicularis oculi, and limb muscles.

Second, a strong correlation was demonstrated between the recovery indices for T1 and for the TOF ratio in the two muscles we examined. This suggests that the faster T1 recovers, the faster the TOF ratio recovers. At the recovery stage, a more rapid decrease in the plasma concentration of a neuromuscular blocking drug is associated with faster T1 recovery. Bartkowski et al. [15] indicated that more rapid decreases in plasma drug concentrations were associated with greater TOF ratios at the same level of T1. The speed of decrease in the plasma concentration of a drug may be associated with the speed of recovery of both T1 and the TOF ratio.

Because movements of the heart and lungs affected the measurements of twitch tensions of the diaphragm in our animal models, the measurements of twitch tensions became more accurate for greater twitch tensions. In our previous study in rats [6], where we stimulated only the right phrenic nerve, the fourth-twitch response to TOF stimuli was small, and the TOF ratio could not be determined accurately. In the current study, we stimulated both phrenic nerves via a bilateral thoracotomy, resulting in larger twitch tensions and more accurate TOF ratios.

In conclusion, our method of simultaneous in vivo evaluation of the TOF ratio in both the diaphragm and the tibialis anterior confirmed significant differences between the two muscles in relationships between first-twitch tension and the TOF ratio, and significant correlations between first-twitch tension and the TOF ratio in each muscle.

Acknowledgments. The author thanks Hironori Itoh, M. D. (Surgical Center, Kanazawa University Hospital) and Munetaka Hirose, M. D. (Department of Anesthesiology, Kyoto Prefectural University of Medicine) for their helpful discussions and comments on the manuscript.

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