

## Characterization of the Train-of-Four Response in Fast and Slow Muscles: Effect of *d*-Tubocurarine, Pancuronium, and Vecuronium

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The *in vivo* cat soleus and gastrocnemius muscles were used to compare isometric contraction strength and the train-of-four ( $T_4$ ) response (2 Hz for 2 s) of two muscle types (fast and slow) during onset of competitive neuromuscular blockade in order to determine the extent of the correlation between twitch depression and  $T_4$  fade. Prior to drug administration the muscles that were studied differed significantly in that the  $T_4$  ratio was 1.0 in the gastrocnemius and only 0.87 in the soleus. Three competitive neuromuscular-blocking agents were compared: *d*-tubocurarine, pancuronium, and vecuronium. *d*-Tubocurarine was found to produce a close correlation between the degrees of twitch strength depression and  $T_4$  for both muscles. However, these muscles demonstrated significantly different  $ED_{50}$  values (105  $\mu\text{g}/\text{kg}$  for gastrocnemius, 150  $\mu\text{g}/\text{kg}$  for soleus). Pancuronium also produced a similar relationship between twitch strength depression and  $T_4$  decrement for each muscle. In this case, however, there was little difference in their  $ED_{50}$  values for twitch depression (11.5  $\mu\text{g}/\text{kg}$  for gastrocnemius, 13  $\mu\text{g}/\text{kg}$  for soleus). The effects of vecuronium were quite different from the other two muscle relaxants. Although vecuronium produced a comparable correlation between twitch tension and  $T_4$  fade in fast muscle, no such relationship was found to exist in slow muscle. Even when the twitch strength was blocked to 18% of control, the soleus  $T_4$  response was depressed to only 75% of control. These results highlight major differences among competitive neuromuscular-blocking agents and suggest multiple sites of action. (Key words: Monitoring; stimulator, nerve. Muscle: gastrocnemius; soleus. Neuromuscular junction. Neuromuscular relaxants: pancuronium; *d*-tubocurarine; vecuronium.)

MEASUREMENT AND MONITORING of the degree of neuromuscular blockade have been achieved by the clinician using some combination of the single twitch, tetanic stimulus, and train-of-four ( $T_4$ ) stimulus to the

adductor pollicis for determination of muscle strength. Several investigators have emphasized the utility of the  $T_4$  as a valid, quantitative measurement of evoked muscle response,<sup>1,2</sup> which not only obviates the need for a control twitch response,<sup>3</sup> but also provides greater sensitivity.<sup>4</sup> In addition, it has been reported that the return of the  $T_4$  ratio correlates well with clinical voluntary tests of muscle strength (*i.e.*, head lift,<sup>5,6</sup> cough,<sup>6</sup> inspiratory force,<sup>7</sup> and peak expiratory flow rate<sup>7</sup>) during recovery from neuromuscular blockade. Others state that the  $T_4$  ratio consistently decreases in close correlation with the single twitch depression during onset of neuromuscular blockade.<sup>8</sup> It is important to note that *d*-tubocurarine has been used frequently in many of these studies. Whether other competitive neuromuscular-blocking agents produce comparable correlations is not entirely certain.

Equally noteworthy, the adductor pollicis, a predominantly fast muscle, has most commonly been utilized to monitor neuromuscular blockade for both investigative work as well as in clinical practice. On the basis of its evoked  $T_4$  and twitch responses and its dose-response characteristics, many of the above conclusions have been reported. The muscles responsible for ventilation and airway maintenance (*i.e.*, diaphragm, intercostals, and vocal cord musculature) are all composed of varying combinations of fast and slow muscle. It is thought that the diaphragm is composed of a heterogeneous mixture of slow and fast fibers on the basis of histochemical and electromyographic analysis.<sup>9</sup> It is unknown whether the information and conclusions obtained from fast muscle can be applied to either slow muscle or to muscles with a mixed composition.

Consequently, this investigation involved a comparison of fast and slow muscle response to a control  $T_4$  stimulus, as well as an evaluation of each muscle's characteristic dose-response to twitch and  $T_4$  ratio produced by each of three agents, *d*-tubocurarine (*d* Tc), pancuronium (PAN), and vecuronium (VEC). Use of an animal model for performance of experimental work enabled isolation of muscles that were predominately either fast or slow in composition.

### Materials and Methods

Cats of either sex, weighing 2 to 3 kg were anesthetized with 75 mg/kg alpha chloralose, iv. Tracheosto-

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mies were performed and the animals were ventilated mechanically with room air by a volume-regulated ventilator. Ventilator settings (*i.e.*, tidal volume,  $V_T$ , and respiratory rate,  $f$ ) were adjusted to maintain arterial blood  $P_{O_2}$ ,  $P_{CO_2}$ , and  $pH$  within normal limits for the cat,<sup>10,11</sup> and were based on a nomogram of  $V_T$  vs. body weight and  $f$ .<sup>†</sup> An intravenous route was established in an external jugular vein, and an intraarterial cannula was attached to a blood pressure transducer. In addition, heart rate, ECG, and body temperature were monitored.

Both the gastrocnemius (fast twitch) and soleus (slow twitch) muscles were isolated, and the sciatic nerve was cut after its dissection from the popliteal space. After the calcaneus was cut, the tendon of each muscle was attached to a separate force transducer using a steel rod in order to record the contractions of each muscle individually. The leg was mounted in a modified Brown Shuster myograph, and the exposed tissues were covered with a thermoregulated mineral oil pool. Drugs were administered intravenously. The cut sciatic nerve was stimulated by means of bipolar platinum electrodes with supramaximal rectangular pulses of 0.2-ms duration at a frequency of 0.2 Hz. A train-of-four stimulus (2 Hz  $\times$  2 s) was interposed prior to the injection of any drug to serve as a control, and also at the point of maximal twitch depression, approximately three minutes following the injection of any drug.

The muscle response was evaluated on the basis of control (pre-drug) twitch strength, and subsequently, dose-response relationships were expressed as either a per cent of control or per cent inhibition. A control (pre-drug)  $T_4$  response (ratio of the fourth twitch height to that of the first) was determined for both fast and slow muscles. In order to assess the relationship between drug dose and  $T_4$  response, each  $T_4$  ratio (post-drug) was expressed as a per cent of the control  $T_4$  ratio. In addition, the relationship between the twitch height and  $T_4$  response was examined following the integration of dose-twitch response and dose- $T_4$  response data.

Best fitting lines for each dose-response curve were determined by the method of least squares. Comparison of the slopes of the fitted lines was made using a  $t$  test.

## Results

### DOSE-RESPONSE RELATIONSHIPS FOR GASTROCNEMIUS (FAST) AND SOLEUS (SLOW) MUSCLE

The effective concentrations of  $d$ Tc, PAN, and VEC required to produce various degrees of twitch strength depression were determined and compared for fast and

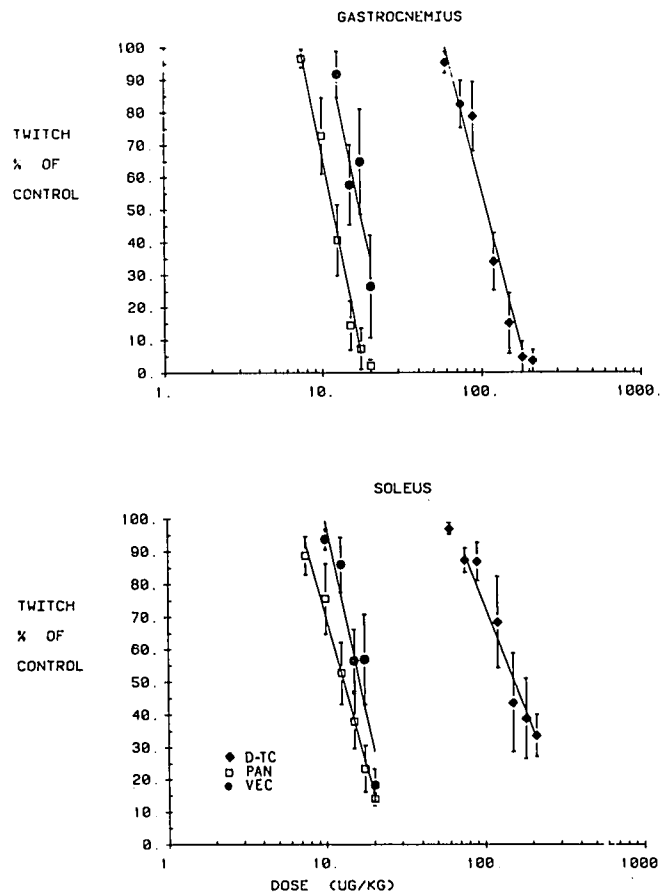


FIG. 1. Dose-response relationship of  $d$ -tubocurarine ( $d$ Tc), pancuronium (PAN), and vecuronium (VEC) on the force of contraction of the cat gastrocnemius and soleus muscles. *Top*: gastrocnemius; *bottom*: soleus. The lines represent the best-fitting lines as determined by least-squares analysis. The values represent the means  $\pm$  SEM,  $n = 5$ . Ordinate: twitch height (% of control). Abscissa: dose of drug ( $\mu$ g/kg intravenously).

slow muscles (fig. 1). The least potent agent was  $d$ Tc, and its  $ED_{50}$  value (dose required for 50% depression of twitch strength) was 105  $\mu$ g/kg, whereas PAN and VEC were considerably more potent with  $ED_{50}$  values of 11.5  $\mu$ g/kg and 18.5  $\mu$ g/kg, respectively. For the soleus muscle,  $d$ Tc was again the least potent agent, with an  $ED_{50}$  of 150  $\mu$ g/kg, while Pan and VEC were nearly equipotent with  $ED_{50}$  values of 13 and 16  $\mu$ g/kg, respectively.

When comparing the twitch response of these muscles, it is evident that  $d$ Tc exhibited a dose-dependent differential effect. For example, 120  $\mu$ g/kg produced a mean twitch depression to  $34 \pm 8$  (SEM) % of control in fast muscle, while slow muscle was blocked to only  $68 \pm 14\%$  of control ( $P < 0.05$ ). This difference in effect widened with higher doses; when 210  $\mu$ g/kg were administered, the mean fast muscle twitch was decreased to  $3.5 \pm 3.5\%$  of control, while slow muscle twitch remained at  $33.5 \pm 6.5\%$  of control ( $P < 0.05$ ). In addi-

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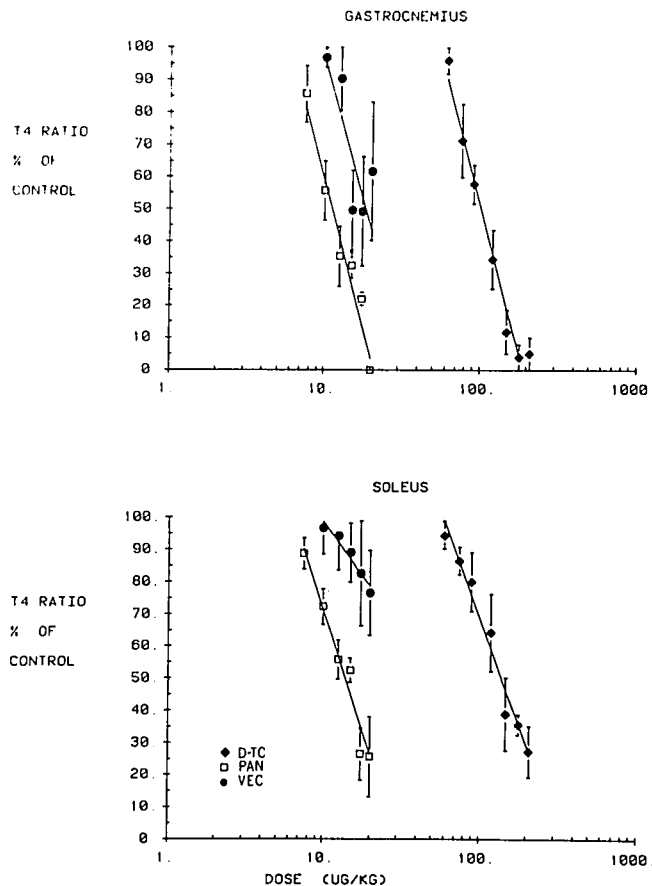


FIG. 2. Dose-response relationship of *d*-tubocurarine (*d*Tc), pancuronium (PAN), and vecuronium (VEC) on the  $T_4$  response of the cat gastrocnemius and soleus muscles. *Top*: gastrocnemius; *bottom*: soleus. The  $T_4$  response was the ratio of the fourth to the first contraction height of each muscle produced by a 2 Hz stimulation of the nerve for 2 s. The lines represent the best-fitting lines as determined by least-squares analysis. The values represent the means  $\pm$  SEM,  $n = 5$ . Ordinate: Train-of-four (% of control). Abscissa: Dose of drug ( $\mu\text{g}/\text{kg}$  intravenously).

tion, the slope of the gastrocnemius *d*Tc curve is statistically different from that of the soleus *d*Tc curve ( $P < 0.01$ ). Neither PAN nor VEC exhibited this preferential blockade of fast muscle and their dose-response curves for fast and slow muscle were not statistically different.

#### CONTROL $T_4$ RATIO OF FAST AND SLOW MUSCLE

The pre-drug evoked  $T_4$  response was found to be different for slow muscle when compared with fast muscle. The gastrocnemius did not exhibit any decrement in the control  $T_4$  ratio, whereas the soleus exhibited a progressive fading of the last three twitches in the train. The control ratios of the second, third, and fourth twitches in relation to the first twitch were  $93\% \pm 1.7$ ,  $89\% \pm 1.7$ , and  $87\% \pm 1.9$ , respectively, in this slow muscle. The second twitch always exhibited the most

pronounced amount of fade followed by a smaller degree of fade in the third twitch, which finally leveled off with little or no fading of the fourth twitch. When this frequency of stimulation was continued beyond the fourth twitch, neither fast nor slow muscle demonstrated any further change in twitch tension development, even after 20 such stimuli. It should be noted that the subsequent calculations of the soleus  $T_4$  response following drug administration took into account this preexistent fade during the control period.

#### $T_4$ RATIO OF FAST AND SLOW MUSCLE FOLLOWING DRUG ADMINISTRATION

The concentrations of each drug which produced various degrees of  $T_4$  decrement also were determined for the gastrocnemius and soleus (fig. 2). Each of the drugs depressed the  $T_4$  response in a dose-dependent fashion. In fast muscle,  $T_4$  dose-response curves reflected a similar relationship in potency between the three drugs comparable to that observed for their respective twitch dose-response curves. In fact, the extrapolated  $ED_{50}$  values for fast muscle  $T_4$  depression ( $100 \mu\text{g}/\text{kg}$  for *d*Tc,  $11 \mu\text{g}/\text{kg}$  for PAN, and  $18 \mu\text{g}/\text{kg}$  for VEC) were nearly identical to the  $ED_{50}$  values for fast muscle twitch depression obtained for each drug. VEC, however, was exceptional in that the  $T_4$  response rarely was depressed beyond 40–50% of control at doses that nearly abolished the twitch.

In slow muscle,  $T_4$  inhibition by *d*Tc and PAN also closely resembled their respective effects on slow twitch depression. The  $ED_{50}$  values of PAN and *d*Tc for  $T_4$  fade, 14 and  $140 \mu\text{g}/\text{kg}$ , respectively, were very similar to their  $ED_{50}$  values for slow muscle twitch depression. In contrast, VEC was fairly ineffective at producing a  $T_4$  fade of the soleus, and even at the highest dose tested, the  $T_4$  ratio remained above 75% of control, and an  $ED_{50}$  value could not be calculated.

#### Discussion

In the clinical setting the evoked  $T_4$  and the twitch response of the adductor pollicis, predominantly a fast muscle, has been used to provide information with regard to the general level of neuromuscular blockade. The results of this study indicate that these are valid measurements for PAN; a given dose of PAN would be expected to produce comparable responses across muscle types. With *d*Tc, however, the prediction of a slow or mixed muscle's behavior, based on a fast muscle response might be subject to interpretation. This conclusion is based on the fact that slow muscle required a higher concentration of *d*Tc than did fast muscle to achieve the same level of neuromuscular blockade. In fact, it has been reported that the muscles of the hand are more sensitive to *d*Tc blockade than the muscles of

respiration,<sup>12</sup> sometimes referred to as "respiratory sparing".<sup>13,14</sup> This has been documented in studies in humans that indicated a greater depression in head lift and hand grip as compared with inspiratory flow rates.<sup>13</sup> Although the relative contribution of other factors (*i.e.*, differences in temperature, blood flow, and motor innervation) could not be ruled out in these earlier studies, the *in vivo* preparation used in this investigation was designed to control for these variables. It is strongly suggested that this differential effect can be explained on the basis of the different sensitivities of fast and slow muscle fibers to this drug. It should be noted that this difference becomes increased in magnitude as the desired level of neuromuscular blockade is achieved.

In reference to VEC, difficulties also may arise from the transference of information obtained from the adductor pollicis to other muscle groups. This is especially pertinent to the T<sub>4</sub> measurement. In contrast, the twitch response seen in fast muscle closely corresponded to that seen in slow muscle. It appears as though twitch measurement is a more reliable indicator of blockade than the T<sub>4</sub> following VEC. Our results also indicate that VEC, unlike *d* Tc, didn't produce a preferential effect on fast muscle. Based on this, VEC would not be expected to produce "respiratory sparing" as observed with *d* Tc. This might be considered to reduce the margin of safety of the drug. However, its short duration and rapid recovery, coupled with its lack of cardiovascular effects, suggest that it is a safe drug with potential advantages.<sup>15</sup>

It would be expected that a given degree of twitch depression would be associated with the same degree of T<sub>4</sub> inhibition among any series of competitive neuromuscular-blocking drugs if they possessed the same mechanism of action. Similar conclusions were reached by Williams *et al.*<sup>16</sup> in their study of the effects of several nondepolarizing neuromuscular-blocking drugs in humans. They observed that the relationship between compound muscle action potential decrement *vs.* twitch depression was not uniform, and thus suggested that there was clear evidence for multiple sites of action among these drugs. Bowman concluded that tetanic fade, which is an exaggeration of T<sub>4</sub> depression, is independent of twitch depression.<sup>17</sup> Thus, our results compliment previous reports which indicate that some agents exhibit preferential prejunctional effects, while other agents appear to be mainly postsynaptic blocking agents. The finding that *d* Tc produced significant T<sub>4</sub> fade correlates with other data that suggest a presynaptic site of action. Our results with VEC also confirm Bowman's conclusion that this agent does not bind to "fade sites" as rapidly as *d* Tc or PAN as compared with its affinity for postjunctional receptors.<sup>17</sup>

We must emphasize, however, that this resistance to fade was most evident in slow muscle following VEC. In addition, it must be stressed further that the two muscle types also responded in a different fashion to T<sub>4</sub> fade before any drug was administered. Therefore, the extrapolation of data across muscle types and between different species may present difficulty in interpretation with regard to mechanism of action or the assessment of muscle function after drug administration.

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