TRAIN-OF-FOUR FADE DURING ONSET AND RECOVERY OF NEUROMUSCULAR BLOCK: A STUDY IN NON-ANAESTHETIZED SUBJECTS

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Muscle fade during train-of-four (TOF) stimulation at 2 Hz is a standard clinical method of estimating degree of neuromuscular block [1,2]. Ali and his co-workers [3] demonstrated a straight line relationship between the height of the first response of the TOF (T1) and the ratio of the fourth (T4) to the first response (TOF ratio) which is now used widely to predict residual neuromuscular block. Early studies of TOF were performed using the mechanomyogram (MMG), but electromyography (EMG) has been used increasingly as an alternative [4]. There is evidence, however, that there may be drug-related disparities between MMG and EMG recording [5]. Also, the relationship between T1 and TOF ratio may differ depending on whether neuromuscular block is being induced or is recovering [6,7]. This finding may have important implications for the clinical use of the T1/T4 relationship.

We report a study of the relationship between T1 and TOF ratio through all stages of onset and recovery of neuromuscular block using simultaneous MMG and EMG recording.

SUBJECTS AND METHODS

We studied six male service volunteers, aged 18–35 yr. All were healthy with no personal or family history of neurological disorder or allergy and no history of trauma to the forearm. The study was conducted in accordance with the recommendations of the World Health Organization guidelines for human studies [8]. Smoking and alcohol intake were restricted for 12 h before any experimental session. The response of adductor pollicis to alcuronium, vecuronium and tubocurarine was studied in each subject at 1-week intervals. The evoked compound action potential (ECAP) and muscle twitch or mechanomyogram (MMG) [9] were recorded simultaneously in the isolated forearm.

SUMMARY

The actions of alcuronium, vecuronium and tubocurarine have been studied in the isolated forearms of six healthy, non-anaesthetized volunteers. The responses of adductor pollicis were measured during onset and recovery of neuromuscular block for each agent. There was a drug-related disparity between mechanomyogram (MMG) and electromyogram (EMG) measurement of the first response of the train-of-four (T1) and of the ratio of the fourth (T4) to the first response (TOF ratio). There were significantly higher T1 values for the EMG than for MMG during alcuronium blockade (P = 0.03). For tubocurarine, however, the relationship was reversed. The relationship between T1 and TOF ratio during onset and recovery of neuromuscular block was a hysteresis. The TOF ratio at 50% T1 was significantly higher during onset than during recovery for all three drugs, measured by MMG or EMG (P < 0.005). Analysis of variance of the differential fade loops failed to show a drug-related effect. We conclude that care should be taken in assuming interchangeability between MMG and EMG measurement of T1. Relationships between T1 and TOF ratio derived during recovery do not necessarily apply during onset and may lead to error in estimating the degree of muscle relaxation.
Isolated forearm technique

The method used was based on that of Feldman and Tyrrell [10]. Each subject was studied lying on a couch at an ambient room temperature of 22 °C. A suitable vein on the dorsum of the right hand was cannulated using a 21-gauge Butterfly needle (Abbott). Needle electrodes for nerve stimulation were inserted subdermally at the ulnar side of the wrist proximal to the skin crease and halfway up the forearm. The upper electrode was sited at least 10 cm proximal to the lower one. A standard anaeroid sphygmomanometer cuff was placed around the arm above the elbow. Felt pad EMG electrodes soaked in saline and covered with conducting gel were mounted into a plastic holder (Medelec type SE40) and strapped over the dorsum of the hand with the positive electrode lying over the first dorsal interosseous space and the second over the second metacarpophalangeal joint.

Trial stimuli of 0.2 ms duration and up to 140 V were given and the electrode positions adjusted to give the maximum single peak value of the ECAP. The potential applied to the stimulating electrodes was adjusted to be at least 30% above the threshold for maximum response, to ensure supramaximal stimulation. The arm with the EMG electrodes was placed into the arm frame assembly of a Myograph 2000 unit (Biometer, Copenhagen) [11]. The thumb was fixed securely into the ring of the strain gauge transducer with gauze swabs to avoid slipping from the correct right-angle position. The arm was fixed by evacuation of the surrounding air splint and the Velcro straps were tightened, taking care not to displace the EMG electrodes.

EMG recording

ECAP signals were amplified and displayed using a Medelec MS6 electromyograph through an AA6 biostable amplifier set to a bandwidth of 8–1.6 Hz. Amplifier gain was set so that the upward (negative) part of the signal covered two or three divisions of the screen. The gain was increased as the signal amplitude decreased after neuromuscular block to maintain this presentation. The linearity of the gain control of the AA6 was verified using the built-in calibration facility. Recordings of ECAP were made on light sensitive paper from the recording oscilloscope of the MS6. During the test period before administration of the neuromuscular blocker, signals were recorded first in a conventional, single sweep x–y mode and thereafter in gated mode which presents the single peak height as a straight line. ECAP amplitude was measured manually from the negative single peak height. At the end of each experiment further recordings of ECAP were made using the conventional x–y plot to compare the final waveform shape with the original. Gross movement artefacts in the EMG electrodes during recording produce a distortion of the ECAP and a consequent error in measuring the single peak height. No results were rejected because of waveform distortion in the 18 recordings made during the study.

MMG recording

MMG signals were recorded graphically and digitally from the Myograph 2000 system. The force–displacement transducer used records reliably over a range of 0–15 kg [11]. The preload applied to the thumb was set to 0.3 kg, using the adjusting ring of the transducer. After evacuation of the splint this value was observed closely during the period of initial stimulation. Stability was gained usually after 2 or 3 min during which the subjects became used to the nerve stimulation and the associated muscle twitches.

Experimental procedure

Each subject was studied double-blind at 1-week intervals when alcuronium 1.5 mg, vecuronium 0.6 mg or tubocurarine 5 mg was used. The order of administration of the drugs was randomized. The doses were determined from pilot studies to provide approximate equivalent relaxation in the isolated forearm, and recovery, within a period of 1 h. Trains-of-four at 2 Hz were generated using the Myotest stimulator which triggers the Myograph recording unit inductively. The Myotest and MS6 units were synchronized by taking the output of the Myotest stimulator and passing it via a Zenner diode to the MS6.

After the isolated forearm arrangement was set up and the response to pulses at 1 Hz was tested, control values of T1 and TOF ratio were measured. Five TOF stimuli were delivered during each 1 min at this time, allowing 10 s between the finish of one train and the start of the next. At time zero the cuff was inflated to 250 mm Hg and blocking drug (diluted in 40 ml of normal saline) was administered i.v. over a period of 40 s. The cuff was left inflated for 3 min, during which time TOF stimuli continued at 10-s intervals.
After 3 min the cuff was released and the same pattern of stimulation continued up to 10 min. After this time, recordings were made at 1-min intervals until return of MMG and EMG to control values or to a maximum time of 50 min after inflation of the cuff.

The isolated forearm technique was tolerated well in all subjects and there were no clinical sequelae, with the exception of occasional slight blurring of vision following release of the cuff and evidence of local histamine release in some subjects to whom tubocurarine was administered.

In order to check the distribution of the saline containing the neuromuscular blocking drug in the forearm, one subject was studied separately using technetium-99 as label. Two different injection sites were examined on two occasions. In the right arm, the $^{99}$Tc-labelled saline was injected through the dorsum of the hand and in the left the injection site was approximately 15 cm proximal to the wrist crease. The tourniquet was inflated for 3 min and the spread of the labelled saline monitored using a gamma camera. Injection through the hand produced acceptably even distribution of the isotope through the forearm, whereas injection through the forearm site produced some concentration in the nearby superficial tissues and uneven distribution. For this reason the hand route was chosen.

**Statistical methods**

MMG and EMG recordings for each agent in the six subjects were analysed using the paired $t$ test and two-way analysis of variance [12]. Correlations between MMG and EMG measurements of TOF ratio and T1 were made across all subjects using a method of weighted averages [13].

**RESULTS**

**Pattern of T1 and TOF ratio responses**

The form of responses obtained from the six subjects studied is illustrated in figure 1, which shows plots of MMG and EMG measurements of T1 and TOF ratio against time from one representative subject (no. 4). Table I shows two variables (T1$_{min}$—the lowest recorded value as a percentage of the control—and T1$_{rec}$—the time for T1 to recover from 25 % to 75 % of the control value) derived from the original data for all six subjects.

There was good correlation between MMG and EMG measurements of T1$_{rec}$ for all three drugs and of T1$_{min}$ for vecuronium ($P < 0.02$), but poor correlation in estimating T1$_{min}$ for alcuronium and tubocurarine ($P > 0.07$).

**Analysis of T1 and TOF ratio responses**

Figure 2 shows scattergrams of all T1 and TOF ratio values recorded by MMG and EMG for three drugs in all subjects. The line shown indicates the theoretical 1:1 relationship. The scattergrams show that there is perceptible deviation from the 1:1 relationship for alcuronium and tubocurarine which is caused by the "bias" of one...
TABLE I. Simplified summary of T1 responses from all subjects. $T1_{\text{mln}}$ is the minimum value recorded as a percentage of the control value and $T1_{\text{rec}}$ the time (min) taken for T1 to recover between 25% and 75% of the control value. Results are shown for MMG and EMG recordings.

<table>
<thead>
<tr>
<th>Subject</th>
<th>MMG</th>
<th>EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1_{\text{mln}} (% of control)</td>
<td>T1_{\text{rec}} (min)</td>
</tr>
<tr>
<td>Alcuronium</td>
<td>1 16 30 11 31 29</td>
<td>12 16 9 5 13.5 9</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>14 16 43 3 37 18</td>
<td>6 8 29 3 48 34</td>
</tr>
<tr>
<td>Vecuronium</td>
<td>1 1 29 18 24 39</td>
<td>1 9 37 14 30 46</td>
</tr>
</tbody>
</table>

FIG. 2. Relationships between T1 by MMG and T1 by EMG, and between TOF ratio by MMG and TOF ratio by EMG from all data points. The straight lines are the theoretical 1:1 relationship. There is visible departure from this relationship in the case of alcuronium and tubocurarine.
Table II. Bias between MMG and EMG measurements (MMG–EMG) of T1 and TOF ratio from all data points. There is significant bias for T1 in the case of alcuronium (P = 0.03)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Vecuronium</th>
<th>Alcuronium</th>
<th>Tubocurarine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T1 TOF ratio</td>
<td>T1 TOF ratio</td>
<td>T1 TOF ratio</td>
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<tr>
<td>1</td>
<td>14.5 21.7</td>
<td>-18.9 -17.7</td>
<td>9.9 9.1</td>
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<tr>
<td>2</td>
<td>-3.9 -7.2</td>
<td>-11.1 -17.5</td>
<td>14.9 1.2</td>
</tr>
<tr>
<td>3</td>
<td>4.5 2.1</td>
<td>-1.4 1.1</td>
<td>0.9 3.4</td>
</tr>
<tr>
<td>4</td>
<td>1.0 1.7</td>
<td>-17.6 -11.5</td>
<td>-5.1 5.7</td>
</tr>
<tr>
<td>5</td>
<td>-5.0 -1.5</td>
<td>-12.2 -5.6</td>
<td>14.8 4.8</td>
</tr>
<tr>
<td>6</td>
<td>-8.3 -7.3</td>
<td>1.0 0.3</td>
<td>13.4 7.8</td>
</tr>
<tr>
<td>Mean</td>
<td>0.5 1.6</td>
<td>-10.0 -8.5</td>
<td>8.1 3.4</td>
</tr>
<tr>
<td>P</td>
<td>0.88 0.72</td>
<td>0.03 0.06</td>
<td>0.06 0.18</td>
</tr>
</tbody>
</table>

Fig. 3. Relationships between T1 and TOF ratio in subject 4. Data from figure 2 for MMG and EMG have been replotted and show a different relationship between T1 and TOF ratio depending on whether measurement is made during onset or recovery of neuromuscular block. The direction of the onset-recovery cycle is given by the symbol at the end of the broken lines: □ = vecuronium; x = alcuronium; ● = tubocurarine.

### Table II

<table>
<thead>
<tr>
<th>Subject</th>
<th>T1 (TOF)</th>
<th>T1 (TOF)</th>
<th>T1 (TOF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5</td>
<td>-18.9</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>-3.9</td>
<td>-11.1</td>
<td>14.9</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>-1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>-17.6</td>
<td>-5.1</td>
</tr>
<tr>
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<tr>
<td>6</td>
<td>-8.3</td>
<td>1.0</td>
<td>13.4</td>
</tr>
<tr>
<td>Mean</td>
<td>0.5</td>
<td>-10.0</td>
<td>8.1</td>
</tr>
<tr>
<td>P</td>
<td>0.88</td>
<td>0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Technique reading higher than the other. No overall bias is obvious for the vecuronium data, except for one line of points all of which were obtained in subject 1. Numerical analysis of the bias for all T1 and TOF ratio results in the six subjects is presented in table II. There is a significant average T1 bias in the case of alcuronium: -10.0 (SD 8.2) (t = -2.99, df = 5, P = 0.03) with 95% confidence interval (95% CI) −18.6 to −1.4. Other analyses of bias failed to achieve statistical significance, although for tubocurarine the average T1 bias of 8.1 (8.4) (95% CI −0.7 to 16.9; t = 2.36, df = 5, P = 0.06) shows a trend in the opposite direction. Only alcuronium showed an indication of average bias for TOF ratio recording (mean −8.5 (8.4) (95% CI −17.3 to 0.3; t = −2.48, df = 5, P = 0.06)), but the result again just failed to achieve significance. If the data in table II are analysed by two-way analysis of variance there is a significant difference between the average MMG–EMG T1 bias for the three drugs (F = 5.92, df = 2, 10, P = 0.02) which is demonstrated most clearly between alcuronium and tubocurarine (95% CI for the difference between the mean bias: 6.3–29.9). Analysis of variance of MMG–EMG TOF ratio values in table II shows no significant drug-related technique bias (F = 3.29, df = 2, 10, P = 0.08).

### Relationship between T1 and TOF ratio

Figure 3 shows the data from figure 1 replotted to give the relationships between T1 and TOF...
ratio for the three drugs studied. In each case the relationship between the two is different during onset and recovery of block, resulting in hysteresis. This feature was seen for all subject-drug combinations, recorded using MMG and EMG. To analyse the factors affecting the loop shape, values of TOF ratio at a T1 value of 50% (the TOF ratio50 value) during onset (O) and recovery (R) of paralysis were selected from each set of curves for each subject. This provided a simple estimate of the difference in fade during these periods and was necessary because of the variable loop shapes produced by the T1 v. TOF ratio plots.

Complete TOF ratio50 values for all subjects are presented in table III. In the three instances marked with an asterisk the subjects did not become paralysed to T1 = 50% and the approximate mid-point of the loop was taken as a comparison point instead. The differences between onset and recovery values of TOF ratio50 (TOF ratio50 (O-R)) measured by MMG and EMG were tested using the paired t test (table III). The onset values were found to be significantly higher than the recovery values in all subjects, with the mean difference ranging between 16.5 and 33.2 (P < 0.005). Two-way analysis of variance of the TOF ratio50 (O-R) values showed considerable between-subject variability and no significant difference between any pair of drugs in contribution to the shape of the loop as determined by TOF ratio50.

**DISCUSSION**

The isolated forearm technique offers a means of investigating the action of neuromuscular blocking drugs in non-anaesthetized subjects. Although introduced several years ago [10], the technique has been relatively neglected as a research tool, although a modification has been suggested for use in clinical neurophysiology [14]. In the study reported here it was used successfully in six subjects during three, weekly sessions in which train-of-four stimuli were delivered regularly for periods of up to 1 h. Simultaneous recording of MMG and EMG showed good correlation between mechanical and electrical recording of the action of adductor pollicis in assessing T1rec for block by alcuronium, vecuronium or tubocurarine and for T1min following vecuronium. There was poor agreement between the techniques in assessing T1min after alcuronium or tubocurarine.

Detailed analysis of all data points revealed a drug-related bias between the two recording methods for T1. We have found that EMG recorded significantly higher values than MMG during alcuronium blockade (P = 0.03), where the mean bias (the average value of MMG – EMG) for the six subjects was –10.0 (95% CI 18.6 to –1.4). For tubocurarine, however, the mean bias was 8.1 (95% CI 0.7 to 16.9), but the result failed to achieve significance (P = 0.06). The bias for alcuronium is in agreement with the finding of Harper, Bradshaw and Healy [5]. There have been a number of other studies which

![Table III. TOF ratios at T1 = 50% of control (TOF ratio50) for all subject-drug combinations. Paired t test analysis shows a significant difference in every case between values during onset (O) and recovery (R) of neuromuscular block measured by both MMG and EMG. * Subjects did not become paralysed to T1 = 50%; mid-point of loop taken for comparison.](http://bja.oxfordjournals.org/).
showed disparity between mechanical and electrical measurements of neuromuscular block, but interpretation is complicated by the different conditions of anaesthesia and test stimulations used. De Jong and Freund [15] compared tetanic tension with EMG single peak height. Following a 5-s tetanus at 40 Hz in patients anaesthetized with nitrous oxide and halothane and paralysed with suxamethonium or decamethonium, they concluded that MMG and EMG could be used interchangeably. Katz [16] found that MMG from thenar and hypothenar muscles paralysed with suxamethonium following 0.1- and 50-Hz stimuli was less than EMG. For tubocurarine the reverse applied, and the author concluded that, wherever possible, MMG and EMG should be studied independently. Epstein and Epstein [17] found wide variations between MMG and EMG from adductor pollicis in patients anaesthetized with nitrous oxide and halothane. More recently, Crul and his co-workers [9] have shown good agreement between integrated EMG and MMG from adductor pollici stimulated with trains-of-four. The present study has also shown that, for both MMG and EMG, there is a significant difference between the relationship of T1 and TOF ratio during onset and recovery of neuromuscular blockade—an effect which might be termed "differential fade". The results confirm, in non-anaesthetized subjects, previous clinical observations [6,7]. We were unable to demonstrate any drug-related influence on differential fade using the TOF ratio method. This may be because of the relative insensitivity of the isolated forearm technique. Analysis of variance showed some between-subject variation, which questions the assumption that the subjects studied were neurophysiologically homogeneous in terms of fibre type mosaic and safety margin [18]. Muscle biopsy would have clarified this point, but was not possible in this volunteer study.

Bowman has suggested that differential fade may be caused by different affinities of non-depolarizing neuromuscular blocking drugs for pre- and post-junctional receptors at the neuromuscular junction [19]. Williams and his co-workers [6] showed that, during onset of paralysis, pancuronium produced the least and gallamine the most fade at 50 % reduction in T1. It has been suggested [Norman, personal communication, 1987] that the newer generation of blockers may show more differential fade than the old. We were unable to show this effect between vecuronium and tubocurarine.

The findings presented here have a number of clinical implications. Care should be taken in extrapolation between MMG and EMG measurement of muscle activity. The relationship between the two techniques has been shown to be influenced by the nature of the blocking agent. The existence of differential fade implies that relationships between T1 and TOF ratio derived during recovery from block should not be used to predict values of T1 during onset.

REFERENCES


