EFFECT OF PRETREATMENT WITH ORAL
PYRIDOSTIGMINE ON SUBSEQUENT ACTIVITY OF
ALCURONIUM IN NON-ANAESTHETIZED SUBJECTS

G. A. TURNER, J. D. WILLIAMS AND D. J. BAKER

SUMMARY
We have studied the effects of alcuronium in 10 healthy, non-anaesthetized volunteers after they had been taking oral pyridostigmine 30 mg 8 hourly. The responses of adductor pollicis were recorded using an isolated forearm procedure (IFP) during onset and recovery of neuromuscular block produced by 1.5 mg of relaxant. Previously-noted disparities between mechanomyogram and electromyogram measurement of the first response of the train-of-four (T1) and the ratio of the fourth (T4) to the first response (TOF ratio) were found in most cases, but were unaffected by pyridostigmine. Pyridostigmine did not affect significantly the overall characteristics of neuromuscular block, but repeated IFP after a placebo unexpectedly produced marginally less depression of T1 and more rapid recovery. The hysteresis relationship between T1 and T4 during onset and recovery of block was confirmed, but was not affected by pyridostigmine. Clinically, the results may indicate that pyridostigmine pretreatment is unlikely to have significant effects on the subsequent use of alcuronium.

KEY WORDS

The carbamate anticholinesterase pyridostigmine bromide is familiar clinically in the treatment of myasthenia gravis and as an antagonist to non-depolarizing neuromuscular blocking drugs in clinical anaesthesia [1]. A newer use is as prophylaxis against irreversible acetylcholinesterase (AChE) inhibition by organophosphate (OP) compounds [2]. These are used widely as pesticides and are a hazard of modern warfare. The prophylactic use of pyridostigmine involves taking 30 mg orally 8 hourly to produce approximately a 40% reduction in the body AChE activity. However, this inhibition may produce problems in patients requiring balanced anaesthesia for intercurrent surgery. Given the conventional use of carbamates to antagonize neuromuscular block, the question arises of possible effects of pretreatment on a subsequently administered non-depolarizing blocking agent. A previous study [3] described the use of an isolated forearm procedure (IFP) which allowed the study of the relationship of the first (T1) response of a train-of-four (TOF) at 2 Hz to the fade of the fourth (T4) compared with the first response in non-anaesthetized subjects. This paper reports a further study where the same technique and analysis have been used to study the effects of pyridostigmine on subsequent neuromuscular block produced by alcuronium.

SUBJECTS AND METHODS
We studied male 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 con-
ducted in accordance with the recommendations of the World Health Organization guidelines for human studies [4]. Smoking and alcohol intake were restricted for 12 h before any experimental session. The response of adductor pollicis to alcuronium 1.5 mg diluted in normal saline 40 ml in the isolated forearm was studied before and after the administration of oral pyridostigmine bromide. The experimental procedure used was identical to that described previously with mechanomyogram (MMG) and electromyogram (EMG) recordings made simultaneously using a Myograph 2000 isometric twitch recorder (Bi-ometer Ltd, Copenhagen) and a Medelec MS6 electromyograph (Medelec Ltd, Old Woking, England) [3]. A fixation period of 3 min was allowed with the cuff inflated after the injection of the neuromuscular blocking drug in the dorsum of the right hand. Recording time zero was taken at the point of injection. Ten subjects were allocated randomly to two groups, either active pyridostigmine or placebo. Each subject was studied during three IFP recorded on study day 1 before taking the drug (IFP1), on study day 2, 2 h after a loading dose of 60 mg (IFP2) and on study day 4, 2 h after taking a final dose of pyridostigmine 30 mg (IFP3). Between the loading and final doses, subjects continued to take pyridostigmine 30 mg 8 hourly. Before each recording venous blood samples were taken for erythrocyte acetylcholinesterase (AChE) analysis using the method of Ellman and colleagues [5].

At each IFP, values were recorded of the first response to TOF stimulation from MMG (MT1) and EMG (ET1) and of the ratio of the fourth to the first response (MT4 and ET4). The ulnar nerve was stimulated supramaximally using stimuli of 0.2 ms duration at 2 Hz. The TOF was repeated at 10-s intervals for 3 min and at 1-min intervals after the release of the cuff up to a maximum of 1 h recording time.

Data in the study were analysed in two ways [6]: comparisons between the three IFP within the active and placebo groups were made using two-way analysis of variance; between-group analysis of the change in response between IFP1 and IFP2, and IFP1 and IFP3 was made using the two-sample t test. Results were considered to be statistically significant if $P \leq 0.05$.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>IFP1</th>
<th>IFP2</th>
<th>IFP3</th>
<th>MT1 (%)</th>
<th>MT1 (min)</th>
<th>ET1 (%)</th>
<th>ET1 (min)</th>
<th>MT4 (%)</th>
<th>MT4 (min)</th>
<th>ET4 (%)</th>
<th>ET4 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6</td>
<td>4.0</td>
<td>3.4</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>7</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>8</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>9</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>5.4</td>
<td>4.2</td>
<td>3.2</td>
<td>24</td>
<td>14</td>
<td>3.6</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.4</td>
<td>3.4</td>
</tr>
</tbody>
</table>
No abnormal reactions were noted from the use of alcuronium in the IFP and there was no clinically detectable neuromuscular block apart from the forearm after release of the cuff. No subjects taking active pyridostigmine reported any adverse symptoms. Subject No. 7 was unavailable for IFP3 recording for reasons unrelated to the study.

Table I shows the measured red cell concentrations of AChE in subjects taking placebo and active pyridostigmine. The values shown are corrected to a standard PCV of 0.45. There was good agreement between the mean active values for IFP2 and IFP3, showing a 40 % reduction in concentration of the enzyme level. The effects of pyridostigmine on alcuronium-induced neuromuscular block were examined by consideration of the maximum block achieved (T1m), the time to reach T1m and the period of recovery from T1m to 90 % of control T1m.

Effects of pyridostigmine on T1m

There was wide variation in the degree of neuromuscular block recorded electrically and mechanically in subjects taking both active and placebo pyridostigmine. Analysis between groups showed that, for ET1m, the differences were not statistically significant. Analysis between active and placebo groups showed that, for MT1m, the differences were significant at IFP3 (P = 0.02), but were not statistically significant at IFP2 (P = 0.05). There was no significant increase in MT1m across IFP1-IFP3 (F = 1.37; df = 1, 12; P = 0.26).

Table II. TOP ratios at 50% of (control T1 - T1m), during IFP1-IFP3 in subjects taking active pyridostigmine (Nos 1-5) and placebo (Nos 6-10). Paired t test analysis shows a significant difference in most cases between values during onset (O) and recovery (R) of alcuronium-induced neuromuscular block measured by both MMG and EMG. Analysis of variance between groups shows no significant effect of pyridostigmine on differential fade. † Subject was unavailable for recording.
significant at IFP2. No differences between groups were significant in the mechanical recordings.

Effects of pyridostigmine on time to 90% recovery

Analysis of variance revealed no significant within-group differences in recovery time between the three IFP for either mechanical or electrical recording. Between the active and placebo groups, there were significant differences for the change between IFP1 and IFP2. The average difference in MT1 recovery time between groups was a reduction of 5.2 min \((t = 6.1; \text{df} = 8; P < 0.001; 95\%\ CI 6.2–13.8\ min)\), composed of an increase of 2.4 min in the active group and a decrease of 7.6 min in the placebo. The corresponding difference in ET1 recovery time was an average reduction of 5 min \((t = 2.7; \text{df} = 8; P = 0.026; 95\%\ CI 1.4–17.4\ min)\).

Effect of pyridostigmine on agreement between MMG and EMG estimations

The difference between MMG and EMG assessment of relaxation parameters was examined as before [3], by analysis of MT1–ET1 and MT4–ET4. In most cases there were negative values of bias. Analysis of variance showed no effect of placebo or pyridostigmine on bias.

Effect of pyridostigmine on differential fade

In almost all recordings in both the placebo and pyridostigmine groups, the relationship between T1 and T4 was a hysteresis. The effect was analysed by taking the T4 value at T1 values during onset (O) and recovery (R) of paralysis which were 50% of control T1–T1min, giving a measure of the effect at the mid-point of the hysteresis curve. Analysis of these values using the paired \(t\) test (table II) shows significant differences between mid-range values of T4 for all IFP within the placebo and active groups. Analysis of variance between the groups shows no significant effect of pyridostigmine on the degree of differential fade.

DISCUSSION

The isolated forearm procedure has been shown to be useful in the study of neuromuscular blocking drugs in non-anaesthetized subjects [3, 7]. Although there may be reservations about the relevance of the performance of these drugs in the isolated forearm to whole body response, the technique is valuable in that it allows direct observation of muscle response without interference from general anaesthesia. The present study examined the effects of pretreatment with pyridostigmine on the subsequent actions of the neuromuscular blocker. Administration of these drugs in this unusual sequence is a possibility in modern military anaesthesia because of the use of pyridostigmine as a prophylactic pretreatment against the effects of subsequent exposure to organophosphates. The use of the drug in this way is based on its ability to combine reversibly with a proportion of the end-plate AChE, producing a complex which is resistant to OP attack [2]. After exposure to OP, which causes irreversible inhibition of non-complexed AChE, the pyridostigmine–AChE complex breaks down, effectively providing an enzyme autotransfusion at the postjunctional membrane. Alcuronium was chosen for study because of its established military use [8]. The 1.5-mg dose of alcuronium used in the IFP was sufficient to produce surgical relaxation in most subjects, although there was wide variation and two subjects did not exhibit paralysis to less than T1 = 25%. Pyridostigmine caused a reduction in red cell concentration of AChE to 60% of normal. This degree of inhibition was not associated with any significant change in the value of T1min. Corrected red cell AChE concentration is a standard enzyme measure [5], but its exact relationship with end-plate AChE is unknown.

Repeated IFP with placebo showed a small but statistically significant effect on the average mechanical minimum T1 response, with a linear increase through the three IFP. This is the opposite of any predicted carry over of non-depolarizing activity of alcuronium. There was no equivalent change associated with pyridostigmine. There was a small reduction in the time taken to reach T1min within the pyridostigmine group which persisted in the between-group analysis. This finding is the opposite of that predicted if pyridostigmine is acting as an antagonist to alcuronium. Between-group analysis also revealed a small reduction in recovery time between IFP1 and IFP2 because of a reduction within the placebo group. The possibility exists that pyridostigmine itself may be causing a degree of depolarization block, but studies using single fibre electromyography [9] have discounted this possibility at the dosage used in this study.

Our previous study [3] showed that for alcu-
ronium in the IFP there was a significant bias of EMG over MMG values of T1. This has been confirmed by the present study which also showed that the bias was unaffected by pyridostigmine. The differential fade effect of T4 with respect to T1 seen previously was confirmed and there was no detectable modification by pyridostigmine. Two current hypotheses for fade [10] are that block of prejunctional cholinergic positive feedback receptors causes a failure of sustained ACh release, or that the open ion channels become blocked by stearic hindrance by the blocking drug. Bowman [10] has suggested that the rate of attachment of the drug at the prejunctional receptor is slower than that postjunctionally. This leads to a gradual onset of fade as ACh release diminishes. The alternative view is that, as the attachment of ACh to the ACh receptor becomes less secure, the channels are influenced more by ACh, which tends to hold the channels open during the TOF. Molecules of the neuromuscular blocking drug then cause channel block.

The effect of pyridostigmine pretreatment may be considered on both these possibilities. In terms of ion channel blocking, by inhibiting AChE, pyridostigmine may produce an increase in the average concentration of ACh at the postjunctional receptors and increase the average channel open-time. In the presence of relaxant, more channel blocking is likely and fade should occur earlier in the IFP. The differential fade would therefore be expected to disappear after pyridostigmine pretreatment. Alternatively, if pyridostigmine has an equal action at the pre- and postjunctional receptors, there may be increased positive feedback leading to increased ACh release. This should cause the rate of attachment of relaxant to the prejunctional receptor to be slowed, leading to a delay in the onset of fade and a shift to the right of the differential fade loop. The finding that differential fade persists after pyridostigmine may therefore favour the prejunctional mechanism, rather than ion channel block as an explanation.

In conclusion, the study has shown that, in the isolated forearm, pyridostigmine pretreatment produced small changes in the action of a neuromuscular blocking drug. Although extrapolation to the whole body is difficult, these results may indicate that the use of pyridostigmine as prophylaxis against OP poisoning is unlikely to produce significant difficulties with the use of neuromuscular blocking drugs in balanced anaesthesia.

REFERENCES