DIFFERING INTERACTIONS BETWEEN HEXAMETHONIUM AND TUBOCURARINE, PANCURONIUM OR ALCURONIUM AT THE NEUROMUSCULAR JUNCTION†

B. J. POLLARD AND A. F. L. VAN DER SPEK

The principal sites of action of the non-depolarizing neuromuscular blocking drugs are the acetylcholine receptors within the neuromuscular junction. The classical view holds that the occlusion of receptors on the post-junctional membrane by drug molecules prevents access of transmitter and, as a result, blocks neuromuscular transmission. However, in addition to the nictinic cholinergic receptors on the post-junctional membrane, the existence of additional acetylcholine receptor subtypes which are situated on the prejunctional nerve endings is supported by present evidence [1]. The reported differences in behaviour of the individual drugs may then be attributed to the result of a different emphasis on the pre- and post-junctional receptors, each agent possessing its own unique profile of activity [2,3]. Prejunctional receptors may have a greater similarity to those cholinergic receptors in autonomic ganglia than to the receptors on the post-junctional membrane of the neuromuscular junction [4]. It has been shown that ganglion blocking agents do prolong the action of non-depolarizing neuromuscular blocking drugs [5, 6] and, in view of the different activity profile of each agent, such an interaction need not be identical for every agent. This study was designed to investigate this hypothesis.

SUMMARY

The action of hexamethonium has been investigated in the rat phrenic nerve–hemidiaphragm preparation, alone and in combination with the neuromuscular blocking agents tubocurarine, pancuronium and alcuronium. Hexamethonium alone in concentrations between 3.55 \times 10^{-3} and \text{7.1} \times 10^{-3}\text{ mol litre}^{-1} produced neuromuscular blockade in a dose-dependent manner. Low concentrations of hexamethonium antagonized the neuromuscular blocking effect of all three neuromuscular blocking drugs, less with tubocurarine than with the other two. Increasing the concentration of hexamethonium produced potentiation of the neuromuscular blocking effect, this being greater with tubocurarine than with either pancuronium or alcuronium. The cholinesterase activity in rat diaphragm homogenates was inhibited by hexamethonium. This inhibition was only significant at concentrations greater than those which resulted in antagonism and cannot, therefore, explain the observed antagonism. The mechanism of these observations is discussed with respect to the known behaviour of a combination of antagonists acting within a receptor system.

MATERIALS AND METHODS

Wistar rats weighing between 250 and 300 g were sacrificed and both hemidiaphragms removed, together with their accompanying phrenic nerves. These were trimmed, attached to carrier assemblies and placed in identical organ baths of 50 ml capacity. Each bath contained Krebs–Henseleit solution with a chemical composition (mmol litre\(^{-1}\)): Na\(^+\) 143, Cl\(^-\) 129, K\(^+\) 5.9, Ca\(^{2+}\) 3.3, Mg\(^{2+}\) 1.2, HCO\(_3\)^{-} 25, SO\(_4\)^{2-} 1.2, H\(_3\)PO\(_4\)^{-} 1.2 and glucose
Carbon dioxide 5% in oxygen was bubbled through the solution and the temperature maintained at 36.5–37.5 °C by means of a thermostatically controlled water jacket. Each hemidiaphragm was stimulated indirectly through the nerve at a frequency of 0.1 Hz, using square wave pulses of 0.2 ms duration and a voltage greater than that required to produce a maximal response. Direct stimuli, using 2-ms square wave pulses, were applied across the muscle at intervals. The resulting single muscle contractions were recorded using an isometric force transducer and displayed on a Grass 79C polygraph. The resting tension on the muscle was maintained constant at 4 g.

Each agent under investigation was added to the bath in a small volume, to obtain the desired final concentration in the bath. The technique of dose accumulation was used, with a contact time of 20 min. The bathing solution was regularly refreshed and each preparation used for the determination of no more than two concentration–response relationships, which were separated by a wash cycle of 40 min incorporating at least six changes of fresh bathing solution. The depression of twitch height was measured at each steady-state response and expressed as a percent reduction from initial control values. Hexamethonium bromide was chosen as the ganglion blocking agent and tubocurarine chloride, pancuronium bromide and alcuronium chloride as the neuromuscular blocking agents.

In the first part of the investigation, the neuromuscular blocking effect of hexamethonium alone was investigated on six different preparations. The threshold concentration below which hexamethonium alone did not produce neuromuscular blockade was determined. A series of four concentrations of hexamethonium was then selected in the range from just below the threshold to about 10% of the threshold, the actual concentrations being determined by the dilution of the stock solution.

In the second part of the investigation a concentration–response relationship was established for each of the three neuromuscular blocking agents alone, and in the presence of the four concentrations of hexamethonium selected in part one. Each preparation was only exposed to one agent, but the hexamethonium concentrations were varied in a random manner. Individual concentration–response relationships were constructed using the technique of least squares regression on a minimum of three points between 15% and 85% twitch height inhibition and the log EC_{25}, log EC_{50} and log EC_{75} determined in each case. The mean log EC_{25}, mean log EC_{50} and mean log EC_{75} were calculated for the six preparations in each group and these means used in the construction of the figures. The slopes of the lines for each combination were also recorded and compared using analysis of variance.

In the light of the results from the above section, it was necessary to examine the effect of hexamethonium on acetylcholinesterase within the rat diaphragm. The method of Ellman and colleagues [7] was used. Acetylthiocholine, hydrolysed to thiocholine by the cholinesterase in rat diaphragm homogenates, liberates thiocholine which, when combined with 5,5'-dithiobis-(2-nitrobenzoic acid), forms a yellow coloured ion, 5-thio-2-nitrobenzoate. This colour change was measured spectrophotometrically at a wavelength of 412 nm and provided a measure of the percent inhibition of cholinesterase activity.

### RESULTS

**Hexamethonium alone**

A dose-dependent depression of indirectly elicited muscle contraction was seen once a concentration of approximtely $3.5 \times 10^{-3}$ mol litre⁻¹ had been exceeded: 100% blockade was achieved with concentrations greater than $7.1 \times 10^{-3}$ mol litre⁻¹ (table I). Hexamethonium could be fully removed from the preparation by the wash cycle used, no carry-over being observed. The hexamethonium concentrations chosen for the second part of the study were $0.59 \times 10^{-3}$, $1.18 \times 10^{-3}$, $1.97 \times 10^{-3}$ and $2.76 \times 10^{-3}$ mol litre⁻¹.

<table>
<thead>
<tr>
<th>Hexamethonium concentration ($\times 10^{-3}$ mol litre⁻¹)</th>
<th>Twitch height inhibition (%) (mean (SEM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.55</td>
<td>12.6 (1.3)</td>
</tr>
<tr>
<td>3.94</td>
<td>28.5 (2.3)</td>
</tr>
<tr>
<td>4.34</td>
<td>43.3 (4.3)</td>
</tr>
<tr>
<td>4.73</td>
<td>61.3 (4.7)</td>
</tr>
<tr>
<td>5.52</td>
<td>84.6 (3.9)</td>
</tr>
<tr>
<td>6.31</td>
<td>93.0 (2.3)</td>
</tr>
<tr>
<td>7.10</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Hexamethonium with tubocurarine, pancuronium or alcuronium

The results are displayed in figures 1-3. The addition of the smallest concentration of hexamethonium (0.59 x 10^{-3} mol litre^{-1}) to alcuronium (fig. 1) or pancuronium (fig. 2) produced a shift to the right of the log concentration-response line, indicating marked antagonism. A doubling of this concentration of hexamethonium to 1.18 x 10^{-3} mol litre^{-1} showed an almost identical effect. As the concentration of hexamethonium was increased further, a shift of the log concentration-response lines back towards the left beyond the control position was seen, indicating a change from antagonism to potentiation. The effect of hexamethonium 0.59 x 10^{-3} mol litre^{-1} (the smallest concentration) on the tubocurarine log concentration-response line was qualitatively similar to its action with the other two agents, although quantitatively not as great (fig. 3). As the concentration of hexamethonium was increased, the log concentration-response line returned to the left, beyond the control line, in a manner similar to that observed with pancuronium and alcuronium. However, this leftward shift (potentiation of neuromuscular blockade) was more marked in the case of tubocurarine than with either pancur-
onium or alcuronium. Figure 4 shows two records for pancuronium in the absence and presence of hexamethonium \(0.59 \times 10^{-3}\) mol litre\(^{-1}\), demonstrating the antagonism with this concentration of hexamethonium. With all three neuromuscular blockers, the presence of hexamethonium resulted in a change in the gradients of the log concentration–response lines (table II). The gradients in the presence of hexamethonium were at all times significantly different from control.

**Hexamethonium and cholinesterase inhibition**

The results are shown in figure 5. At concentrations of hexamethonium less than \(1.2 \times 10^{-3}\) mol litre\(^{-1}\) there was very little effect on the activity of the cholinesterase from the rat diaphragm. Above this concentration, hexamethonium produced a marked dose-dependent inhibition of cholinesterase, the highest concentration of hexamethonium \((8 \times 10^{-3}\) mol litre\(^{-1}\)) resulting in a mean inhibition of 55.2%.
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Table II. Gradients of mean log concentration–response lines. Compared with gradient in absence of hexamethonium: **P < 0.01; ***P < 0.001

<table>
<thead>
<tr>
<th>Hexamethonium concentration (^{-3}) ((\times 10^{-3}) mol litre(^{-1}))</th>
<th>0</th>
<th>0.59</th>
<th>1.18</th>
<th>1.97</th>
<th>2.76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>224</td>
<td>137***</td>
<td>147***</td>
<td>119***</td>
<td>104***</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>254</td>
<td>186**</td>
<td>182**</td>
<td>139***</td>
<td>118***</td>
</tr>
<tr>
<td>Alcuronium</td>
<td>332</td>
<td>275**</td>
<td>221***</td>
<td>129***</td>
<td>92***</td>
</tr>
</tbody>
</table>

DISCUSSION

Hexamethonium has been shown to depress indirectly elicited muscle contraction without an effect on direct muscle stimulation. This suggests that it has an inhibitory action at the neuromuscular junction, an observation which has been reported previously [5, 8–10]. The concentration range for this action obtained in the present study is in agreement with the findings of those previous workers.

The action of each of the neuromuscular blocking agents was first antagonize and then potentiated by hexamethonium. The three agents examined were selected on account of their differing actions at the neuromuscular junction, tubocurarine having a large pre-junctional effect, while the other two have very similar activity profiles and act primarily on the post-junctional acetylcholine receptors [2]. Inspection of the log concentration–response lines reveals that the antagonism was stronger for pancuronium and alcuronium than for tubocurarine while, conversely, the potentiation was greater with tubocurarine than with either of the other two. The interactions of hexamethonium with pancuronium and alcuronium were similar. This implies that the observed differences may be related to their receptor actions within the neuromuscular junction. Such a difference between the behaviour of the individual neuromuscular blockers in their interaction with other drugs has not previously been documented.

Considering the observed antagonism, it has been suggested [10] that cholinesterase inhibition may be a principal factor. At the concentrations of hexamethonium at which we found antagonism to be the most marked (less than \(1.2 \times 10^{-3}\) mol litre\(^{-1}\)), inhibition of cholinesterase was minimal. It seems, therefore, unlikely that this is an important factor in the antagonism of neuromuscular blockade produced by hexamethonium, rendering our findings at variance with other workers in this respect. When a weak antagonist (hexamethonium) is added to a strong antagonist (tubocurarine, pancuronium or alcuronium) an alteration to the dynamic equilibrium within the junction occurs, resulting in the displacement of some molecules of the latter agent. Therefore, a reduction in effect, or antagonism [11] might be expected, and it would seem likely that this could explain the mechanism behind the observed antagonism; namely, a direct interaction between an antagonist (neuromuscular blocker) and a partial agonist (hexamethonium). Furthermore, such an interaction would be expected to produce a shift in the log concentration–response line in a non-parallel manner [12], which is in agreement

![Figure 5. The inhibition of cholinesterase from rat diaphragm produced by hexamethonium (mean and SEM bars).](https://academic.oup.com/bja/article-abstract/61/4/419/286594/164419286594)
with our observed changes in the log concentration–response line gradients throughout (table I).

Potentiation of the blockade from all three neuromuscular blocking agents resulted from higher concentrations of hexamethonium. The acetylcholine receptors on the pre-junctional region have been reported as having a greater similarity to those in autonomic ganglia than do the receptors on the post-junctional membrane [4]. It is tempting, therefore, to suggest that herein lies the mechanism for this observation: hexamethonium reinforcing the action of the non-depolarizing neuromuscular blocking drugs by additionally blocking the pre-junctional receptors. An examination of the presently accepted activity profiles of the neuromuscular blocking agents does not, however, support this theory. The addition of a pre-junctional action from hexamethonium would be expected to reinforce pancuronium or alcuronium-induced blockade (principally post-junctional in action) more than tubocurarine-induced blockade (pre- and post-junctional in action). The converse was seen, making an interaction at the post-junctional receptors seem more likely. This lends support to a previous study in which it was suggested that the interaction of hexamethonium and tubocurarine was post-junctional in nature [10].

In conclusion, we have demonstrated that hexamethonium interacts with three individual, non-depolarizing neuromuscular blocking agents in different ways, and this has been illustrated by the construction of a series of log concentration–response lines. The findings serve to emphasize the dissimilarity of action of the neuromuscular blocking drugs, although from this study additional light cannot be shed upon the nature of the interactions described at receptor level over and above that already known [13]. It is clear that phenomena which may be attributed to the individuality of each myoneural blocker at the neuromuscular junction are not confined to interactions between the neuromuscular blockers themselves, but extend to interactions with other drugs.

REFERENCES