ANTAGONISTS TO NEUROMUSCULAR BLOCKING AGENTS
An Experimental Study
BY
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SUMMARY
The antagonism between tubocurarine and alcuronium chloride on the one hand and neostigmine, physostigmine, edrophonium and ambenonium on the other have been investigated. A quantitative measure of antagonism was obtained on the rat diaphragm preparation and it was found that in general the antagonists were more effective against alcuronium chloride than against tubocurarine. The antagonist drugs potentiated the action of acetylcholine on frog rectus muscle. The muscarinic action of ambenonium was less apparent than that of the other drugs but it caused severe reduction in amplitude of cardiac contraction and arrest in 50 per cent of the preparations. It is concluded that ambenonium is unlikely to prove a satisfactory drug for further investigation in man.

Neostigmine is almost universally used, in the United Kingdom at least, as an antagonist to tubocurarine. It serves the purpose adequately and cases of neostigmine-resistant curarization are very rare, much rarer indeed than was suggested by the correspondence which followed the original paper on the subject (Hunter, 1956), for at that time the other possible causes of this syndrome were not fully appreciated. There have, however, been occasional fatalities following the administration of neostigmine and it is necessary, in any case, to give large covering doses of atropine to minimize the muscarinic side effects. An antidote to curarization which causes fewer muscarinic side effects in man than does neostigmine would, therefore, be clinically useful.

Other agents, such as, for example, edrophonium (Hunter, 1952) and tacrine (Hunter, 1965), have been tested but neither is capable of reversing the more profound levels of curarization.

The drug ambenonium (WIN-8077) was developed primarily for the treatment of myasthenia gravis and has already been used to some extent for this purpose (Osserman, 1959). In some experimental animals it seemed to possess less direct muscarinic activity than neostigmine (Lands et al., 1955). It certainly showed less muscarinic side effects, in equally effective doses, than did neostigmine in the treatment of myasthenia gravis (Schwab, 1963). Detailed studies of its antagonism to neuromuscular blocking agents were, however, lacking and it seemed appropriate to investigate this drug more fully as a possible substitute for, and an improvement on, neostigmine.

METHODS
Demonstration of antagonism.
This was done using two methods. The head-drop doses of tubocurarine was determined in a group of rabbits by slow intravenous injection of the drug from a tuberculin syringe, in a concentration such that about 5 minutes was required for the development of head-drop. A week later the animals were given ambenonium intravenously in a dose of 0.1 mg/kg and then the head-drop dose tubocurarine determined again.

The protection of mice from lethal doses of tubocurarine and gallamine by ambenonium was also investigated by the intravenous injection of the neuromuscular blocking agent with and without the antagonist in the same syringe.

Comparison with other antagonists.
The use of pA₂ values and the Arunlakshana and Schild plot. The comparison of antagonists has been greatly simplified by the concepts of the A₂ dose and the pA₂ value (Schild, 1947). This concept, though difficult to comprehend, may be more readily understood by reference to figure 1. In this figure is plotted the percentage
reduction in the amplitude of the lever movement produced in a rat diaphragm by the administration of tubocurarine to the bath in which the preparation sits (vertical axis). On the horizontal axis is plotted the dose of tubocurarine. The result is a series of log dose-response curves with the normal S-shape.

![Graph of log dose-response curves](https://example.com/graph.png)

**FIG. 1**
The effect of tubocurarine with and without neostigmine on the force of the contraction of a rat diaphragm produced by stimulating the phrenic nerve. (The small horizontal lines marked "x" indicate how the extent of the displacement of the response curve to the right was measured.)

- **x axis**: Dose of tubocurarine moles/10^-/l.
- **y axis**: Per cent reduction in amplitude.

- **Curve 1**: Tubocurarine alone.
- **Curve 2**: Preparation pretreated with 0.33 x 10^- M neostigmine.
- **Curve 3**: Preparation pretreated with 0.66 x 10^- M neostigmine.
- **Curve 4**: Preparation pretreated with 1.32 x 10^- M neostigmine.

If a small amount of neostigmine is added to the bath, a larger amount of tubocurarine will be required to produce the same amount of inhibition of the lever movement and a new log dose-response curve can be plotted. This new curve will be parallel to that for the original experiment but will be displaced to the right (fig. 1). The extent of the displacement will vary with the dose of neostigmine. Indeed it is possible to plot a whole family of curves for different doses of neostigmine in which the extent of the displacement to the right will bear a relationship to the amount of neostigmine in the bath. The A, dose of neostigmine is that which makes it necessary to use twice the amount of tubocurarine to produce the same reduction in lever movement. Or to put it another way, it is the amount which increases the logarithm of the dose of tubocurarine required to produce the same effect by 0.3010 (the logarithm of 2 to the base 10).

It is not altogether convenient to obtain the dose of neostigmine which will exactly double the dose of tubocurarine necessary to produce the same effect but it has been shown for reasons related to the law of mass action, which are too complicated to discuss here but are presented in detail by Arunlakshana and Schild (1959), that if the log (dose ratio—1) is plotted against the logarithm of the concentration of the antagonist this gives a straight line (fig. 2) and if the antagonism is competitive, the slope of the line is —1. The dose ratio in this context is the ratio of the initial concentration of tubocurarine to the concentration which produces the same effect. If we denote the dose ratio by (x) the ratio of the logarithms involved is

\[
\log(x - 1) = \log(2) \cdot \text{pA}_2.
\]

**FIG. 2**
Derivation of the pA, value.

- **x axis**: Negative logarithm of concentration of neostigmine in bath.
- **y axis**: 

\[
\text{pA}_2 = \frac{\text{Dose of tubocurarine effective in presence of neostigmine}}{\text{Dose of tubocurarine equally effective without neostigmine}} - 1.
\]

i.e. \( \log(\text{dose ratio } (\equiv x) - 1). \)
response in the presence of a given concentration of neostigmine. Under these circumstances the $pA_2$ value which is the logarithm of the $A_2$ value (just as the pH is the logarithm of the hydrogen ion concentration) can be obtained where the straight line slope intercepts the X axis. Non-competitive antagonists may still give a straight line plot, but the slope will not be $-1$ though the $pA_2$ value can still be derived as described above.

Strictly speaking, these concepts are not applicable to anticholinesterase antagonism of neuromuscular blocking agents (which themselves act as antagonists to acetylcholine) as readings were not taken at equilibrium and antagonism at more than one receptor is involved. However, the use of these concepts to measure potency differences, and show possible mechanistic differences is allowable provided that it is not used to suggest what these mechanisms are.

Thus, this method of measuring antagonism of tubocurarine and alcuronium chloride by four anticurare drugs was applied to the rat diaphragm preparation method of Bülbirng (1946) set up in the apparatus used by Starmer and Thomas (1961). The anticurare agents used were neostigmine, ambenonium, physostigmine and edrophonium.

A similar investigation was carried out on the isolated frog rectus muscle set up in a tissue bath according to the technique of Chang and Gaddum (1933). In this method the frog rectus muscle was stimulated by the addition of small quantities of acetylcholine to the bath. The muscle fibre membrane of this preparation is sensitive as a whole to the transmitter; there are no endplates as there are with mammalian muscle fibres. One of four anticurare agents was added to the bath and allowed to act for half an hour. It now required substantially less acetylcholine to produce a contraction of the same height. Log dose-response curves were constructed for acetylcholine in which the dose of acetylcholine added to the bath was plotted against the extent of the lever movement it produced. Similar log dose-response curves were constructed for the effect of acetylcholine in the presence of various concentrations of the anticurare agents. From the family of curves it was possible to obtain a mathematical expression for the extent to which each of the anticurare agents potentiated acetylcholine. An exactly similar plot of log (dose ratio $-1$) against concentration of agent was obtained from these families of log dose-response curves, and from this plot a $pP_2$ value was obtained in exactly the same way as the $pA_2$ values were determined for the antagonism between tubocurarine, alcuronium chloride and the drugs antagonistic to them. Finally the $pP_3$ values were determined once more in the presence of a single dose each of tubocurarine and diallylnortoxiferine added to the bath with the anticurare agent.

Study of side effects.

The most important side effect of neostigmine in clinical practice is muscarinic activity. This was tested by counting the rate of the Langendorff isolated rat heart perfused with double glucose Krebs solution oxygenated with 95 per cent oxygen and 5 per cent carbon dioxide (Pleuvry, 1967). A mechanical counting system was employed (Wislicki, 1964).

RESULTS

Rabbit head-drop.

The dose of tubocurarine which produced head-drop in a group of five rabbits was 0.23 mg/kg (range 0.15–0.29). After the administration of ambenonium (0.1 mg/kg) the dose increased to 0.39 mg/kg (range 0.29–0.48).

Mouse protection.

Ambenonium protected mice against lethal doses both of tubocurarine and gallamine triethiodide. Relevant results are to be found in table 1.

Rat diaphragm.

Neostigmine, ambenonium, edrophonium and physostigmine were all effective antagonists of tubocurarine and alcuronium chloride, the effectiveness against the latter relaxant being greater. The actual $pA_2$ values for the different antagonists will be found in table II, column 1.

The Arunlakshana and Schild plots (see method) gave straight lines over the range of concentrations tested. When tubocurarine was the agonist the slopes of these plots (see table II) indicated a division of the anticurare agents into

N.B.—All drug concentrations are expressed as molar concentration in the in-vitro experiments.
two groups, neostigmine and physostigmine on the one hand and ambenonium and edrophonium on the other. However, when alcuronium chloride was the agonist only edrophonium showed a significantly different slope from that of the other agents.

**Frog rectus.**

In this preparation all the anticurare agents potentiated acetylcholine but ambenonium was the most potent and the most persistent as indicated by the time necessary to wash it from the preparation. This potentiation was also noted on the preparations treated with tubocurarine and alcuronium chloride (see table III). It is interesting to note that ambenonium was significantly more potent as a potentiator of acetylcholine in the presence of the blocking agents than without them. The slopes of the Arunlakshana and Schild plots showed the division of the four drugs into two groups in the presence of both neuromuscular blocking agents (cf. rat diaphragm results) and alone. The division, as before, was neostigmine and physostigmine on the one hand and ambenonium and edrophonium on the other.

**Isolated rat heart.**

There was little evidence of muscarinic side effects in mice given ambenonium. In the rabbits which were protected against the head-drop of tubocurarine, however, salivation was a feature.

In the isolated rat heart, neostigmine produced the most marked bradycardia of the four anticurare drugs studied, and a log dose-response curve could be obtained. Figure 3 shows this and the log dose-response curve for acetylcholine for comparison. Edrophonium was the next most potent, followed by physostigmine, but the latter was not sufficiently soluble to allow construction of a log dose-response curve.

Ambenonium had no consistent effect on heart rate in the concentrations used, and the effect that did appear was usually tachycardia. However,

### Table I

<table>
<thead>
<tr>
<th>Myoneural blocker</th>
<th>Dose (mg/kg)</th>
<th>Antagonist</th>
<th>Dose (mg/kg)</th>
<th>Mice injected</th>
<th>Mice survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallamine</td>
<td>3.5</td>
<td>—</td>
<td>—</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Gallamine</td>
<td>3.5</td>
<td>Ambenonium</td>
<td>0.02</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Gallamine</td>
<td>3.5</td>
<td>Neostigmine</td>
<td>0.20</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>Ambenonium</td>
<td>0.013</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>Ambenonium</td>
<td>0.025</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>Ambenonium</td>
<td>0.05</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>Neostigmine</td>
<td>0.10</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.175</td>
<td>Neostigmine</td>
<td>0.20</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table II

**Assessment of anticurare activity on the rat phrenic nerve diaphragm preparation.**

<table>
<thead>
<tr>
<th>Anticurare agents</th>
<th>pA, values (mean and SE)*</th>
<th>A&amp;S† slopes (mean and SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tubocurarine</td>
<td>Diallylnortoxiferine</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>5.80 (±0.07)</td>
<td>6.16 (±0.03)</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>4.97 (±0.05)</td>
<td>5.33 (±0.05)</td>
</tr>
<tr>
<td>Edrophonium</td>
<td>4.84 (±0.14)</td>
<td>5.36 (±0.05)</td>
</tr>
<tr>
<td>Ambenonium</td>
<td>5.95 (±0.10)</td>
<td>6.52 (±0.08)</td>
</tr>
</tbody>
</table>

* All means and standard errors were based on six experiments.
† A&S = graph of log (dose ratio − 1) plotted against the negative logarithm of the molar concentration of anticurare agent (Arunlakshana and Schild, 1959).
severe reduction in amplitude and cardiac arrest without previous change in rate occurred in 50 per cent of the preparations. The onset of irregularities of rhythm often heralded cardiac arrest.

**DISCUSSION**

This investigation has shown that ambenonium is capable of reversing myoneural block due to tubocurarine, alcuronium chloride and gallamine, and where comparisons of potency were made it was the most potent in this respect.

The mechanism by which this reversal is obtained may be different from that of neostigmine and physostigmine. This is indicated by the differences in the Arunlakshana and Schild plot slopes.

The possibility that acetylcholine had already produced a maximum response in the frog rectus preparation need not seriously be entertained because a log dose-response curve did not flatten off. In any case this particular investigation involved the cumulative administration of the drugs.

Since test preparations from two different species were used and similar results obtained, it is unlikely that these results were a peculiarity of the preparation used. As pointed out in the

<table>
<thead>
<tr>
<th>Potentiator</th>
<th>Alone</th>
<th>Tubocurarine</th>
<th>Alcuronium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neostigmine</td>
<td>5.93 (± 0.046)</td>
<td>6.01 (± 0.058)</td>
<td>6.29 (± 0.051)</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>5.45 (± 0.031)</td>
<td>5.28 (± 0.051)</td>
<td>5.59 (± 0.040)</td>
</tr>
<tr>
<td>Edrophonium</td>
<td>6.20 (± 0.161)</td>
<td>5.60 (± 0.069)</td>
<td>6.43 (± 0.079)</td>
</tr>
<tr>
<td>Ambenonium</td>
<td>7.34 (± 0.063)</td>
<td>8.45 (± 0.130)</td>
<td>8.74 (± 0.168)</td>
</tr>
</tbody>
</table>


**FIG. 3**

The effect of anticholinesterases on the isolated perfused rat heart.

A = acetylcholine for comparison.

N = neostigmine.

E = edrophonium.

P = physostigmine.
method section, the methods used in this investigation can only indicate possible differences and give no indication of what these differences might be.

Cardiac effects.

Besides being a more potent anticholinesterase agent than neostigmine, ambenonium also has the advantage of causing little bradycardia in the isolated heart. The bradycardia caused by the anticholinesterase agents on the isolated heart is probably due to direct muscarinic activity. The relative anticholinesterase potency of the four drugs is the reverse of that observed in induction of bradycardia in the isolated heart. Ambenonium has been shown to be, by far, the most potent anticholinesterase agent of the four agents tested (Blaber, 1963); thus it is unlikely that anticholinesterase activity could explain the bradycardia.

It should be noted that the isolated heart is not influenced by either the sympathetic or parasympathetic nervous system so the results reported may not be the same for an innervated heart.

However, the results so far discussed indicate that the drug ambenonium might be useful as an anticholinesterase agent in man, but its basic action on the heart noted in this study must make this doubtful. In addition, Schwab (1963) using the drug for myasthenic patients comments that despite the lower frequency of muscarinic side effects observed with ambenonium its longer duration of action made overdosage more dangerous.

ACKNOWLEDGEMENT

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REFERENCES


