Potencies of Neuromuscular Blocking Agents at the Receptors of the Atrial Pacemaker and the Motor Endplate of the Guinea Pig

Stanley Lee Son, M.B.,* and Barbara E. Waud, M.D.†

Drug receptor dissociation constants (Kd) were determined for four neuromuscular relaxants at the cardiac pacemaker as well as at the motor endplate. The ratios Kmuscarin/Kneuromuscular were found to be high for d-tubocurarine and dimethyltubocurarine, 264 and 136, respectively. Thus, interaction at muscarinic sites would occur only with large doses of these drugs. In contrast, the ratios were low for pancuronium and gallamine, 5.3 and 2.4, respectively. Hence, the concentrations of these drugs needed for clinical neuromuscular blockade would occupy appreciable fractions of cardiac muscarinic receptors, and thus might produce vagal blockade and thereby produce the tachycardia seen clinically with these two agents. (Key words: Neuromuscular junction; Heart, atria, tachycardia; Neuromuscular relaxants, dimethyltubocurarine; Neuromuscular relaxants, d-tubocurarine; Neuromuscular relaxants, gallamine; Neuromuscular relaxants, pancuronium.)

Although the principal effect of neuromuscular blocking agents is to antagonize the action of acetylcholine at the neuromuscular junction, these drugs may also antagonize acetylcholine at other cholinergic receptor sites. In particular, the muscarinic receptors of the cardiac pacemaker may be affected, thereby causing tachycardia. As a first step in seeking an explanation for the tachycardia seen with drugs such as gallamine and pancuronium, the potencies of neuromuscular blocking agents were compared in isolated heart and skeletal muscle preparations from the guinea pig.

Methods

Guinea pigs of unselected sex weighing between 250 and 500 g were killed by a blow to the head. The heart or lumbral muscle was rapidly removed.

The spontaneously contracting right atrium was suspended in 50 ml Krebs’ solution at 36 C and bubbled with 95 per cent oxygen and 5 per cent carbon dioxide. Atrial rate was recorded by attaching the atrial appendage to a force transducer. Addition of carbachol to the bath slowed the atrial rate. The amount of slowing was expressed as a percentage of the initial heart rate and used to generate cumulative dose–response relationships (fig. 1).

After careful dissection to avoid producing an injury potential, the lumbral muscle was suspended in Krebs’ solution at 36 C, bubbled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. Addition of carbachol to the bath resulted in depolarization of the motor endplate, which was recorded extracellularly by the “moving fluid” electrode technique of Fatt.† The muscle was scanned repetitively until a temporal peak of endplate depolarization had been reached, usually in three to four minutes. Then the carbachol was washed out. Doses of carbachol, spaced 20 minutes apart (as this had previously been found to give adequate recovery periods3), were used to obtain the dose–response relationship.

In both series of experiments, after control dose–response curves had been obtained, the neuromuscular blocking agent under test was added to the bath and dose–response relationships for carbachol again determined. Finally, the neuromuscular blocking agent was washed out and recovery of the preparation demonstrated by return of the dose–response curve to control levels (fig. 1).

The neuromuscular blocking agents, d-tubocurarine,† dimethyltubocurarine,§ gallamine,¶ and pancuronium,** were examined. The pure salts were dissolved in 0.9 per cent sodium chloride solution to make stock solutions.

Statistical Analysis

The dose–response relationships obtained were fitted by an iterative method3 to the logistic function:

\[
E = \frac{A^p}{A^p + [K_d(1 + B^q/K_0)]^q}
\]

Where:

- E = response at concentration A
- M = maximal response
- A = concentration of agonist (carbachol)

* Assistant Professor of Anaesthesia, Harvard Medical School; Associate in Anaesthesia, Peter Bent Brigham Hospital, Boston, Massachusetts.
† Professor of Anaesthesia, University of Massachusetts, Medical School, Worcester, Massachusetts.

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Address reprint requests to Dr. Lee Son: Department of Anaesthesia, Peter Bent Brigham Hospital, 721 Huntington Avenue, Boston, Massachusetts 02115.

† Matheson, Coleman and Bell.
§ K & K.
¶ Davis and Geck.
** Generously supplied by Dr. H. A. Strade of Organon, Inc.
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P = slope of the dose–response curve

\[ \text{K}_A = \text{ED}_{50} \text{ of carbachol in absence of neuromuscular blocking agent} \]

B = concentration of neuromuscular blocking agent

\[ \text{K}_B = \text{dissociation constant of the neuromuscular blocking agent (the value of interest)} \]

Q = a measure of the stoichiometry of the antagonist–receptor reaction; Q corresponds to the slope of a Schild plot

This function was first fitted with a separate P for each curve, then with a common P. From an analysis of variance of the scatter about the two sets of fitted values, parallelism of curves was tested. When parallelism was demonstrated, a reliable estimate of \( \text{K}_B \) and its standard error were then available from the fitting process with the common slope.

Results

In both series of experiments, the effect of addition of neuromuscular blocking agents was to shift the dose–response curve to the right in the usual “parallel” fashion consistent with competitive kinetics. Estimates of \( \text{K}_B \) for both preparations are given in Table 1. The \( \text{K}_B \) ratios for \( d \)-tubocurarine and dimethyltubocurarine were high, the values being 264 for the former and 136 for the latter (Table 1). In contrast, the ratios were low for pancuronium and gallamine, the values being 5.3 and 2.4, respectively.

Discussion

The dissociation constants (\( \text{K}_B \)) for these neuromuscular blocking agents at the motor endplate of guinea-pig skeletal muscle are in agreement with values reported by Lu. For gallamine, \( \text{K}_B \) (\( \text{guinea-pig} \)) was found to be 1.1 \( \mu \text{M} \), similar to the value of 1.86 \( \mu \text{M} \) (\( \text{pA}_2 = 5.73 \)) reported by Brown and Crout. For pancuronium, \( \text{K}_B \) (\( \text{rat} \)) was found to be 0.13 \( \mu \text{M} \), very different from the value of 3.63 \( \mu \text{M} \) (\( \text{pA}_2 = 5.44 \)) reported by Saxena and Bonta, a difference for which we have no explanation. For atropine, Saxena and Bonta reported a \( \text{pA}_2 \) of 6.8, i.e., \( \text{K}_B = 158 \text{ nM} \). For atropine, our \( \text{K}_B \) (\( \text{rat} \)) was 0.6 \( \text{nM} \), agreeing well with a value of 0.4 \( \text{nM} \) reported by Thron and Waud. Brown and Crout reported a \( \text{pA}_2 \) of 8.57 (\( \text{K}_B = 2.69 \text{ nM} \)) for atropine at the guinea-pig atrium, again in line with our value. Paton and Rang found a \( \text{K}_B \) of 1.1 \( \text{nM} \) and Arunlakshana and Schild reported a \( \text{pA}_2 \) of 8.8 (i.e., \( \text{K}_B = 1.58 \text{ nM} \)) for atropine at muscarinic receptors in guinea-pig ileum. Thus, the lower value for pancuronium would seem more reliable.

The marked differences in \( \text{K}_B \) ratios for various neuromuscular blocking drugs are interesting when clinical usage is considered. Because of the magni-

![Fig. 1. Example of typical dose–response relationships obtained with guinea-pig atrial preparation. Abscissae: Concentrations of carbachol. Ordinates: Changes in heart rate expressed as percentages of initial heart rate. ○ control; ■ dimethyltubocurarine, 10 \( \mu \text{M} \); ▲ dimethyltubocurarine, 20 \( \mu \text{M} \); ● dimethyltubocurarine, 40 \( \mu \text{M} \); ○ after washout. The muscle experiments yielded results analogous to those in the heart experiments and similar to those published previously from this laboratory.]

### Table 1. Drug–Receptor Dissociation Constants (\( \text{K}_B \)) in Guinea-pig Preparations

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \text{K}_B ) (( \mu \text{M} ) ± SE)</th>
<th>Number of Observations</th>
<th>( \text{K}_B ) (( \mu \text{M} ) ± SE)</th>
<th>Number of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d )-Tubocurarine</td>
<td>28.2 ± 3.4</td>
<td>3</td>
<td>0.107 ± 0.005</td>
<td>12</td>
</tr>
<tr>
<td>Dimethyltubocurarine</td>
<td>7.5 ± 0.53</td>
<td>3</td>
<td>0.0552 ± 0.0063</td>
<td>5</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.13 ± 0.008</td>
<td>2</td>
<td>0.0247 ± 0.0014</td>
<td>11</td>
</tr>
<tr>
<td>Gallamine</td>
<td>1.1 ± 0.05</td>
<td>3</td>
<td>0.458 ± 0.031</td>
<td>5</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.0063 ± 0.00045</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
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\( \text{pA}_2 = \log (10)^{-1} \) = \( -6 \) = 6.

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\( \text{pA}_2 \) is the negative logarithm of the concentration of a drug that produces a given response.

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tude of the margin of safety at the neuromuscular junction, to 90 to 95 per cent of the receptors at the motor endplate must be occluded before twitch response is completely abolished. Thus, a concentration of neuromuscular blocking agent equal to 9–19 times its \( K_B \) is necessary. For \( d \)-tubocurarine and dimethylytubocurarine the \( K_{Batroium}/K_{Bthunder} \) ratios are so large that concentrations of these drugs needed to produce muscle relaxation clinically will still have little effect on the atrial pacemaker. However, for gallamine and pancuronium the \( K_{Batroium}/K_{Bthunder} \) ratios are small enough that concentrations of these drugs used for muscle paralysis approach the range that might be expected to produce vagal blockade at the atrial pacemaker.

Hughes and Chapple studied the effects of non-depolarizing neuromuscular blocking agents on peripheral autonomic mechanisms in cats. They found that with pancuronium and gallamine, neuromuscular paralysis was accompanied by vagal blockade. However, during neuromuscular paralysis by \( d \)-tubocurarine and dimethylytubocurarine, the cholinergic receptors in the heart could still be activated directly by the application of metacholine. Though obtained in a less well controlled system, these observations agree well with our results and indicate that both sets of observations are not peculiar to a single species. This suggests that the present results are likely to extend across species lines to man.

Previously it had been suggested that the tachycardia seen with gallamine reflected a release of endogenous catecholamines analogous to that seen with tyramine. However, a comparison of the concentrations required for the adrenergic action (about 100 \( \mu M \)) with the \( K_B \) of 1 \( \mu M \) seen for the cholinergic effect indicates that the latter is a much more likely explanation for the tachycardia observed.

The clear-cut differences in \( K_B \) ratios among neuromuscular blocking agents correlate superbly with the known tendencies or lack thereof to produce cardioacceleration clinically. It appears that interference with vagal activity may be contributing partially to the tachycardia seen with pancuronium and gallamine.

References
1. Fatt D: The electromotive action of acetylcholine at the motor end-plate. J Physiol (Lond) 111:408–422, 1950