A Vagolytic Action of Neuromuscular Blocking Agents at the Pacemaker of the Isolated Guinea Pig Atrium

Stanley Lee Son, M.B.,* and Douglas R. Waud, M.D., D.Phil.†

To examine the basis of tachycardia seen clinically with some neuromuscular blocking agents, the potencies of d-tubocurarine, dimethyltubocurarine, gallamine, and pancuronium in antagonizing the effects of vagal stimulation on the guinea pig atrial pacemaker were determined and expressed as an EDₙ₀ for vagal blockade. These EDₙ₀ values were compared with the respective potency values of these agents at the motor endplate. This comparison showed that in clinical doses, gallamine and pancuronium may reach levels that produce vagal blockade. Comparison with atropine indicated that the vagolytic action of the neuromuscular blocking agents was not attributable to receptor occlusion, but reflected instead an action on the vagus nerve itself. (Key words: Neuromuscular junction; Heart, atria; Neuromuscular relaxants, dimethyltubocurarine; Neuromuscular relaxants, d-tubocurarine; Neuromuscular relaxants, gallamine; Neuromuscular relaxants, pancuronium; Parasympathetic nervous system, atropine; Parasympathetic nervous system, vagus.)

In clinical use, neuromuscular blocking agents may produce tachycardia. When dissociation constants of these agents at muscarinic receptors of the cardiac pacemaker and at the motor endplate were compared, the relative potencies of pancuronium and of gallamine were such that appreciable blockade of receptors at the atrial pacemaker would accompany neuromuscular blockade. However, the actual fraction of receptors that must be occupied before a diminution of vagal function would occur is not known. Vagal transmission is well known to be less sensitive to block by an atropine-like action than is the response to exogenous acetylcholine. This implies that a large fraction of the receptors must be blocked to interfere with vagal transmission, but the actual receptor block required can be found only by experimental measurement. Therefore, the potencies of d-tubocurarine, dimethyltubocurarine, pancuronium, and gallamine in blocking the effects of vagal stimulation on the atrial pacemaker were measured directly for comparison with dissociation constants at the motor endplate. In addition, comparable measurements were made for atropine, not only for pharmacologic comparison with the neuromuscular blocking agents, but also because pancuronium has been described as “atropine-like.”

Methods

Guinea pigs of unselected sex weighing between 250 and 500 g were killed by a blow to the head. The right vagus nerve was located in the neck, traced into the thorax, and removed in continuity with the right atrium. The nerve and atrium were then suspended in a bath containing 50 ml Krebs’ solution at 36 C, which was bubbled with oxygen, 95 per cent, and carbon dioxide, 5 per cent. One end of the atrium was fixed and the other attached to a force transducer to record spontaneous contractions of the atrium. The vagus nerve was passed through a ring electrode for stimulation.

The vagus was stimulated by a 5-sec train of supramaximal pulses of 5 msec duration at frequencies of 1, 2, 5, 10, 20, and 30 Hz to produce graded degrees of atrial slowing, from which a control frequency–response curve was constructed. Next, the test drug was added and the response to vagal stimulation redetermined. Finally, the drug was washed out for 30 min and the response to vagal stimulation determined once again. Full recovery on washout was always obtained when one of the neuromuscular blocking agents had been tested. Full recovery was not practical when atropine was used because of the long duration of action of the drug.

The effect of all test drugs was to decrease the slowing produced by vagal stimulation (fig. 1 gives an example with pancuronium). Graded concentrations of neuromuscular blocking agents and atropine were applied so that a dose–response curve for each agent could be generated. Drugs included in the study were d-tubocurarine, dimethyltubocurarine, pancuronium, gallamine, and atropine. Hexamethonium was also examined to confirm that the system contained a ganglionic synapse.

* Assistant Professor of Anaesthesia, Harvard Medical School; Associate in Anesthesia, Peter Bent Brigham Hospital, Boston, Massachusetts.
† Professor of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts.
Accepted for publication August 15, 1977. Supported by grant NS 12255 from N.I.N.D.S.
Address reprint requests to Dr. Lee Son: Department of Anaesthesia, Peter Bent Brigham Hospital, 721 Huntington Ave., Boston, Massachusetts 02115.

0003-0022/78/0300—0191$00.60 © The American Society of Anesthesiologists, Inc.
The effect of the neuromuscular blocking agents and atropine on the response to stimulation was to shift the frequency–response curve to the right and downward. To permit objective analysis of the shift of the curve, a specific model was used as a basis for a maximum likelihood statistical fitting process. The model used for fitting the curves was the commonly used logistic function:

\[ E = M \left( \frac{A^p}{A^p + K^p} \right) \]

where: \( E \) represents the effect—slowing of atrial rate, \( M \) the maximal response, \( K \) frequency producing a half maximal slowing, \( A \) frequency of stimulation and \( P \) determines the slope of the frequency–response curve.

This function was chosen simply because it provides a reasonable fit to a sigmoidal curve. With this model, a shift to the right can be incorporated through increasing \( K \) by a multiplicative factor; a shift of the curve downward can be introduced by decreasing \( M \) by a scale factor. Since the neuromuscular blocking agents exerted both effects, we chose to use a product of two such parameters. Thus, the specific final forms were:

\[ E = M \frac{A^p}{A^p + (K_B)^p} \quad \text{and} \quad E = M \frac{A^p}{A^p + K^p} \]

for the control curve and the curve in the presence of the blocking drug, respectively. The parameters \( \alpha \) and \( \beta \) were introduced in forms such that values of unity corresponded to no drug effect, while values of zero corresponded to maximal effect. These two parameters were combined in the form \( 1 - \sqrt{\alpha \times \beta} \) to give an index that progressed from 0 to 1 as drug effect increased. These values of \( 1 - \sqrt{\alpha \times \beta} \) were plotted against concentrations of the blocking agent to yield sigmoidal dose–response curves for vagal blockade (fig. 2 gives examples). The \( E_D_{50} \) of these curves, which we shall call a \( V_B_{50} \) (vagal blocking \( E_D_{50} \)) was then used as a measure of potency.

All experimental observations were fitted to the appropriate model by an iterative least-squares maximum-likelihood technique, analogous to that previously described.⁴

Use of the specific mathematical models described above need not imply mechanistic significance. The models are used solely to provide the basis for objective statistical analysis. The experimental design is such that drugs are compared by a null method (i.e., concentrations producing matching responses are compared), so the final interpretation is not sensitive to precise choice of model.

**Results**

Vagal stimulation produced both graded and reproducible degrees of slowing of atrial rate so that frequency–response curves were easily generated (fig. 1). As expected, the vagal effect was blocked by hexamethonium (7 to 10 \( \times \) \( 10^{-5}\)M) and atropine.

The \( V_B_{50} \)'s for the four neuromuscular blocking agents and for atropine are compared with previously obtained¹ antagonist–receptor dissociation constants \( K_B \) (lumbrical) and \( K_B \) (atrium) (table 1). The index
VAGOLYTIC ACTION OF NEUROMUSCULAR BLOCKING AGENTS

Fig. 2. Dose–response relationships of the neuromuscular blocking agents and atropine. *Abbreviations:* Concentration of neuromuscular blocking agent and atropine. Ordinates: Index of vagal blockade = 1 - √α x β.

K_B can be interpreted as the molar concentration of antagonist blocking half the receptor pool; thus, the lower the K_B, the more potent the antagonist. The potency ratios for VB_{50}/K_B (lumbrical) show that pancuronium and gallamine block the vagus at doses reasonably close to neuromuscular blocking doses, whereas d-tubocurarine and dimethyltubocurarine require relatively large doses. In turn, pancuronium and gallamine block the vagus [VB_{50}/K_B (atrium)] at concentrations lower than expected from their atrial K_B values.

Discussion

This study shows that the vagal blocking activity of pancuronium or gallamine occurs at concentrations likely to be present clinically. A concentration from nine to 19 times the neuromuscular K_B of a competitive agent is needed to produce the 90–95 per cent receptor occupancy associated with neuromuscular block. When the VB_{50} values for each drug were compared with their corresponding dissociation constants at the motor endplate, d-tubocurarine and dimethyltubocurarine were found unlikely to block the vagus in concentrations needed for muscle relaxation, because the ratios VB_{50}/K_B (lumbrical) were high, i.e., 396 and 318, respectively. By contrast, a ratio of 6.24 for gallamine indicates that at clinical dose levels, this agent is likely to produce antagonism to the effect of vagal stimulation on the atrial pacemaker. Similarly, the VB_{50}/K_B (lumbrical) ratio of 21.5

Table 1. Potency Estimates in Guinea Pig Atrial Preparations

<table>
<thead>
<tr>
<th></th>
<th>K_B (Lumbrical)* (µM)</th>
<th>K_B (Atrial)* (µM)</th>
<th>VB_{50}† (µM)</th>
<th>VB_{50}/K_B (Lumbrical)</th>
<th>VB_{50}/K_B (Atrial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>—</td>
<td>.00063</td>
<td>.0116 (9, .002)</td>
<td>—</td>
<td>18.41</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>.0247</td>
<td>.13</td>
<td>.532 (8, .104)</td>
<td>21.54</td>
<td>4.09</td>
</tr>
<tr>
<td>Gallamine</td>
<td>.458</td>
<td>1.1</td>
<td>2.86 (6, .375)</td>
<td>6.24</td>
<td>2.60</td>
</tr>
<tr>
<td>Dimethyltubocurarine</td>
<td>.0552</td>
<td>7.5</td>
<td>17.6 (6, 3.27)</td>
<td>318.8</td>
<td>2.35</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>.107</td>
<td>28.2</td>
<td>42.4 (5, 9.15)</td>
<td>396.26</td>
<td>1.50</td>
</tr>
</tbody>
</table>

* Values from Lee Son and Waud.† Figures in parentheses are numbers of preparations and standard errors.
for pancuronium suggests that pancuronium might antagonize the effect of vagal stimulation, especially when large doses are used, or during peak levels following intravenous injection. These observations are in line with those made previously on the basis of a comparison of potencies expressed as dissociation constants at these two sites, and extend the conclusions of the preceding paper in this series.\(^1\) It is now clear that not only are receptors blocked at clinical dose levels, but the block is extensive enough to result in interference with vagal action.

The action of atropine is to block the vagus nerve at the nerve–atrial junction; the ratio \(\text{VB}_{40}/K_B\) (atrium) for atropine can therefore be used to calibrate our system. Thus, the value of 18.4 obtained indicates how extensive a pure receptor block must be to interfere with the effects of neural stimulation. When each of the neuromuscular blocking agents was compared in a similar manner, the \(\text{VB}_{40}/K_B\) (atrium) was found to be much less than 18.4; that is, the neuromuscular blocking agent had an effect beyond that explicable by an atropine-like action.\(^\S\)\(^\S\) For \(d\)-tubocurarine, the ratio was 1.5—presumably reflecting the known ganglionic action of this agent. Dimethyltubocurarine, gallamine and pancuronium do not have appreciable ganglionic blocking actions.\(^7\)^8

\(^\S\)\(^\S\) The argument is somewhat complex; to illustrate consider pancuronium. The ratio \(\text{VB}_{40}/K_B\) was 4.09. From conventional receptor kinetic analyses,\(^4\) this means that at the \(\text{VB}_{40}\) the fraction of receptors blocked was 0.8. However, the value of 18.4 for atropine indicates 0.95 of the receptors must be blocked to reduce the vagal response to half. Therefore, pancuronium had not blocked enough receptors to explain the vagal block. In other words, the vagal block must reflect some effect from another mechanism.

References

1. Lee Son S, Waud BE: Potencies of neuromuscular blocking agents at the receptors of the atrial pacemaker and the motor endplate of the guinea pig. Anesthesiology 47: 34–36, 1977

Downloaded From: http://anesthesiologypubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931495/ on 11/28/2018