FAILURE OF NEOSTIGmine TO PREVENT TUBOCURARINE NEUROMUSCULAR BLOCK IN THE ISOLATED ARM

S. A. FELDMAN AND S. AGOSTON

SUMMARY
Neostigmine failed to modify the development of neuromuscular block in the presence of a high local concentration of tubocurarine. Only when the concentration of tubocurarine was reduced, and a suitable concentration gradient established between the receptor area and the plasma, did neostigmine increase the rate of recovery from the action of non-depolarizing neuromuscular blocking agents.

Baraka (1967) observed that neostigmine was unable to antagonize the neuromuscular block produced by tubocurarine if the plasma concentration of antagonist was greater than 2.0 μg ml⁻¹ and caused only slow antagonism when the plasma concentration was greater than 1.0 μg ml⁻¹. This effect was confirmed by Baraka in 1977. Katz and Katz (1975) described the relative ineffectiveness of agonist drugs to antagonize pancuronium block when the recovery of the indirectly elicited twitch was less than 20% of control unless a considerable time had elapsed since the administration of the last dose of pancuronium. This present study using the isolated arm technique (Feldman and Tyrrell, 1970) allowed us to study the effectiveness of various doses of neostigmine in antagonizing tubocurarine neuromuscular block both when the plasma drug concentration was high and when it was low.

METHOD
The "isolated arm" technique described by Feldman and Tyrrell in 1970 was used for these experiments. An arterial tourniquet was inflated on the upper arm to isolate it from the general circulation just before a dose of tubocurarine 2 mg diluted in 40 ml of saline was injected rapidly into a vein on the dorsum of the hand. The tourniquet was released at a set time after completion of the injection (tourniquet time). Neuromuscular conduction was monitored by stimulating the ulnar nerve at the wrist with a square-wave supramaximal stimulus of 0.2 ms at 0.5 Hz and recording the tension produced in the adductor pollicis muscles using a Statham UC3 load cell preloaded to 50 g.

In these experiments the dose of tubocurarine used was standardized at 2 mg, irrespective of the size of patient (52–82 kg), as the distribution volume was limited to one arm only.

Various doses of neostigmine were used to promote an increased agonist activity in these experiments. It was always mixed with atropine 1.2 mg to minimize muscarinic side-effects. When neostigmine was administered systemically the dose was standardized at 0.05 mg kg⁻¹, but when it was given in the isolated arm, doses from 0.25 to 1.5 mg in 40 ml of saline were used.

The original experiments were carried out on both conscious volunteers and patients anaesthetized with 1% halothane with nitrous oxide and oxygen before surgery, without any discernable difference in the results. However, in order to make our conclusions as clinically relevant as possible, only the results from the anaesthetized patients are included in this paper. The consent of patients to the procedure was obtained in all cases.

(1) In the control group of three patients, tubocurarine 2 mg diluted in 40 ml of saline was injected into the isolated arm and the tourniquet deflated after 2 min (fig. 1).

(2) Three experiments were carried out administering neostigmine and atropine systemically 5 min before the injection of the tubocurarine into the isolated arm and the tourniquet deflated after 2 min (fig. 1).

(3) Eight experiments were performed using mixtures of tubocurarine 2 mg and neostigmine 0.25 mg, 0.5 mg (fig. 2A), 1.0 mg or 1.5 mg together in the solution used in the isolated arm with a tourniquet time of 2 min. To control the
possible difference in onset time of neostigmine and tubocurarine, one additional experiment used a tourniquet time of 1 min and another experiment used a tourniquet time of 5 min.

(4) Following the establishment of neuromuscular block using tubocurarine 2 mg in the isolated arm with a 2-min, tourniquet time, four investigations were carried out administering neostigmine 0.05 mg kg\(^{-1}\) systemically at 25% recovery of neuromuscular transmission (fig. 2 c).

RESULTS

Amount of block
The extent of the neuromuscular block achieved in the isolated arm was not significantly altered by systemic pretreatment with neostigmine 0.05 mg kg\(^{-1}\) compared with control (table I: 92.7±9.2% block compared with 94.3±4.1). Mixing neostigmine 0.25 mg, 0.5 mg, 1.0 mg or 1.5 mg with tubocurarine 2 mg in the isolated arm produced 92.5±3.6% (table II) compared with control of 94.3±4.1% when tubocurarine was administered alone in the isolated arm. Reducing the tourniquet time to 1 min and increasing it to 5 min did not influence the amount of neuromuscular block produced (93.0±3.6% compared with control of 94.3±4.1%). Compared with the control group there was no statistically significant difference in the amount of block achieved in the three groups receiving neostigmine using the Mann–Whitney test for biological significance.

Recovery of neuromuscular transmission
The recovery index (Feldman, 1972)—the time taken for the twitch response to recover from 25% to 75% of its control height—was used to compare the recovery rates from the tubocurarine block in all the experiments. This gives a good comparative index of the rate of recovery of neuromuscular transmission.
NEOSTIGMINE AND ISOLATED ARM TUBOCURARINE BLOCK

Table I. Effect of pretreatment with neostigmine 0.05 mg kg\(^{-1}\) on neuromuscular block produced by tubocurarine 2 mg

<table>
<thead>
<tr>
<th></th>
<th>Max. block (%)</th>
<th>Time to 25% recovery (min)</th>
<th>Time to 75% recovery (min)</th>
<th>Time to full recovery (min)</th>
<th>Recovery index 25% to 75% (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
<td>8</td>
<td>24</td>
<td>38</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>6</td>
<td>18.5</td>
<td>27</td>
<td>13.5</td>
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<tr>
<td></td>
<td>98</td>
<td>8</td>
<td>21.5</td>
<td>32</td>
<td>13.5</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>82</td>
<td>2.5</td>
<td>12.0</td>
<td>16.0</td>
<td>9.5</td>
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<td></td>
<td>98</td>
<td>15.0</td>
<td>26.5</td>
<td>36.0</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>9.5</td>
<td>21.0</td>
<td>28.5</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Table II. Effect of neostigmine 0.5 mg injected concurrently with tubocurarine 2 mg or of neostigmine 0.05 mg kg\(^{-1}\) injected at 25% recovery, on the neuromuscular block produced by tubocurarine. * 1-min tourniquet time; † 5-min tourniquet time

<table>
<thead>
<tr>
<th></th>
<th>Max. block (%)</th>
<th>Time to 25% recovery (min)</th>
<th>Time to 75% recovery (min)</th>
<th>Time to full recovery (min)</th>
<th>Recovery index 25% to 75% (min) Mean</th>
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</thead>
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<tr>
<td>Neostigmine with tubocurarine 2 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg</td>
<td>90</td>
<td>2.0</td>
<td>14</td>
<td>&gt;14</td>
<td>12.0                  10.25</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>4.0</td>
<td>12.5</td>
<td>22</td>
<td>11.5                  11.7</td>
</tr>
<tr>
<td>0.5 mg</td>
<td>94</td>
<td>6.0</td>
<td>17.5</td>
<td>&lt;20</td>
<td>12.5                  12.25</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>2.6</td>
<td>14.5</td>
<td>18</td>
<td>12.0                  12.25</td>
</tr>
<tr>
<td>1.0 mg</td>
<td>92</td>
<td>4.0</td>
<td>16.5</td>
<td>24</td>
<td>12.5                  12.55</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>5.0</td>
<td>17.0</td>
<td>27</td>
<td>12.6                  12.6</td>
</tr>
<tr>
<td>1.5 mg</td>
<td>95</td>
<td>5.5</td>
<td>18.0</td>
<td>&lt;25</td>
<td>12.5                  12.55</td>
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<td>3.2</td>
<td>14.8</td>
<td>20</td>
<td>12.6                  12.6</td>
</tr>
<tr>
<td>0.5 mg*</td>
<td>92</td>
<td>5.0</td>
<td>18.5</td>
<td>27</td>
<td>13.5                  13.5</td>
</tr>
<tr>
<td>0.5 mg†</td>
<td>95</td>
<td>8.0</td>
<td>21.5</td>
<td>34</td>
<td>13.5                  13.5</td>
</tr>
<tr>
<td>Neostigmine at 25% recovery</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.6                    2.5</td>
</tr>
</tbody>
</table>

Compared with the control patients to whom no neostigmine was administered, patients receiving 0.05 mg kg\(^{-1}\) i.v. 5 min before tubocurarine 2 mg in the isolated arm demonstrated a quicker recovery of neuromuscular conduction (table I: 10.5 ±1.73 min compared with 14.0 ±1.8 min in the control). This is significantly more rapid (one-tailed Mann-Whitney test).

Adding neostigmine 0.25–1.5 mg with the 2 mg of tubocurarine had a small effect on the recovery index (table II compared with table I: 12.3 ±1.7 min compared with 14.0 ±1.8 min in control).

The administration of neostigmine 0.05 mg kg\(^{-1}\) at 20% recovery of neuromuscular block had a dramatic effect on reversal. The 25% to 75% recovery time was reduced to 4.7 ±1.75 min compared with 14.0 ±1.8 min recovery index in the control group (table II compared with table I).

DISCUSSION

The object of this investigation was to repeat in vivo experiments previously carried out by others to study agonist/antagonist relationships using in vitro preparations. As it was impossible to achieve similar equilibrium conditions in patients, the isolated arm technique was used to simulate in vitro conditions.

When tubocurarine 2 mg in 40 ml of saline is injected i.v. in the isolated arm, a concentration gradient of drug is achieved which results in sufficient drug passing to the receptor site to cause more than 90% neuromuscular block. By the end
of the 2 min tourniquet time a state of "pseudo equilibrium" is established and, so long as the tourniquet is inflated, the block remains constant. The presence of this concentration of drug in the plasma determines the degree of neuromuscular block irrespective of the concentration of acetylcholine. The injection of neostigmine 0.25–1.5 mg with the tubocurarine in the isolated arm in these experiments must have increased the available acetylcholine at the receptor site, but failed to influence the amount of block produced by tubocurarine 2 mg. This is difficult to reconcile with in vitro experiments studying the interaction of agonist and antagonist agents at the neuromuscular junction, which have demonstrated consistent antagonism of the block. In vitro, larger doses of agonist are required to prevent neuromuscular block in the presence of greater concentrations of antagonist drug. This log-dose relationship has been demonstrated for a variety of agonist and antagonist drugs in vitro (Jenkinson, 1960; Lu, 1970; Waud, Cheng and Waud, 1973). However, if there are two factors controlling the recovery of neuromuscular transmission following the administration of an antagonist drug—the dissociation rate constant and the concentration gradient between receptor and blood—then, although increased acetylcholine production will facilitate the dissociation of the drug–receptor combination, the absence of a favourable concentration gradient will prevent the drug leaving the receptor area. As a result, the amount of block produced will be unaffected by the presence of neostigmine.

Once the tourniquet is released a favourable concentration gradient will be established and the effect of neostigmine in increasing the rate of drug–receptor dissociation will be revealed. This is most obvious at peak blood concentrations of neostigmine. The effect is most pronounced when the neostigmine is administered in high doses during recovery from neuromuscular block and less effective when administered in the same dose 5 min before the establishment of tubocurarine neuromuscular block. Even after the administration of small doses of neostigmine (0.25–1.5 mg mixed with tubocurarine), once the cuff was released and the blood concentration of tubocurarine reduced to subparalytic values, faster recovery was apparent in most patients although the mean was not significantly different from the mean control recovery index.

If one envisages a simple competition between acetylcholine and a non-depolarizing relaxant for receptor sites at the neuromuscular junction, then it should be possible to antagonize the action of a given concentration of antagonist in the biophase by suitably increasing the dose of agonist, provided sufficient time is allowed for equilibrium to be established. There have been several case reports indicating that even in the relatively stable pharmacokinetic conditions produced in anephric patients to whom a relative overdose of gallamine has been inadvertently administered, complete antagonism of the neuromuscular block by neostigmine is impossible (Fairley, 1950; Montgomery and Bennett-Jones, 1956; Feldman and Levi, 1963).

This is in agreement with Baraka's contention (1967, 1977) that the majority of cases of irreversible curarization are a result of high concentrations of drug in the plasma. Agoston, Feldman and Miller (1979) have suggested that, in vivo, the greater the diffusion gradient between the receptor site and the plasma, the more rapid will be the recovery from neuromuscular block. If the gradient is high (as in the isolated arm following release of the tourniquet) recovery is rapid. If the gradient is minimal (as after continuous infusion of antagonist) recovery is prolonged. This paper takes this concept one stage further by demonstrating the failure of neostigmine to prevent the development or modify the extent of the neuromuscular block produced by subparalytic concentrations of tubocurarine. This suggests that, unless a suitable gradient exists between the receptor and the plasma, allowing drug molecules to leave the receptor area once they have been displaced from the receptor site, it will be impossible to antagonize the neuromuscular block even by large doses of neostigmine.

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REFERENCES


NEOSTIGMINE AND ISOLATED ARM TUBOCURARINE BLOCK

LA NEOSTIGMINE N'EMPECHE PAS LE BLOCAGE NEUROMUSCULAIRE PAR LA TUBOCURARINE DANS LE BRAS ISOLE

RESUME
La néostigmine n'a pas été en mesure de modifier l'évolution du blocage neuromusculaire en présence d'une forte concentration locale de tubocurarine. Ce n'est que lorsque l'on réduisit la concentration de tubocurarine et que l'on établit un gradient de concentration approprié entre la zone réceptrice et le plasma que la néostigmine eut pour effet d'accroître le rythme de récupération contre l'action des agents non dépolarisants de blocage neuromusculaire.

NEOSTIGMINE NICHT FÄHIG, TUBOCURARINDUZIERTE NEUROMUSKULÄRE BLOCKADE IM ISOLIERTEN ARM ZU VERHINDERN

ZUSAMMENFASSUNG
Neostigmine war nicht fähig, die Entwicklung einer neuromuskulären Blockade im Beisein einer hohen örtlichen Konzentration von Tubocurarin zu beeinflussen. Nur als die Konzentration von Tubocurarin reduziert und eine angemessene Konzentrationskurve zwischen Rezeptorbereich und Plasma erreicht wurde, konnte Neostigmine die Erholung von der Wirkung der nicht depolarisierenden neuromuskulären Blockierungsmittel beschleunigen.

LA NEOSTIGMINA NO LLEGA A PREVENIR EL BLOQUEO NEUROMUSCULAR POR LA TUBOCURARINA EN EL BRAZO AISLADO

SUMARIO
La neoestigmina no llegó a modificar el desarrollo del bloqueo neuromuscular frente a una alta concentración de tubocurarina. Sólo cuando se redujo la concentración de tubocurarina y se pudo establecer una gradiente adecuada de la concentración entre la zona receptora y el plasma, la neoestigmina aumentó el ritmo de recuperación a raíz de la acción de los agentes bloqueadores neuromusculares no-depolarizantes.