IN VITRO COMPARISON BETWEEN THE NEUROMUSCULAR AND GANGLION BLOCKING POTENCY RATIOS OF ATRACURIUM AND TUBOCURARINE

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SUMMARY

Results from in vitro experiments, using the hypogastric nerve—vas deferens preparation, and the phrenic nerve—hemidiaphragm preparation of the guineapig, have been used to determine the separation between the neuromuscular and ganglionic blocking effects of atracurium and tubocurarine. Regression lines were used to calculate the concentrations of each drug (99% confidence limits) which would produce a 50% blockade (EC50) of ganglionic and neuromuscular transmission. The equipotent molar ratio, using EC50 values, for ganglionic/neuromuscular blockade was 48 for atracurium and 9.4 for tubocurarine.

The tendency of drugs which produce neuromuscular blockade also to reduce ganglionic transmission is recognized. The ganglion blocking potency of tubocurarine and hexamethonium have been shown to be similar (Paton, 1959; Birmingham and Hussain, 1980) and furthermore tubocurarine may produce some ganglion blockade in a dose range similar to that required to produce neuromuscular block (Bowman, 1982). A ganglion blocking action may impair the autonomic reflexes occurring during surgical operations (Burstein et al., 1950) and this may be associated with hypotension (McDowell and Clark, 1969). The separation between the dose of drug which produces muscle relaxation and the dose which produces ganglion blockade is therefore an important consideration before the clinical introduction of a new muscle relaxant.

Atracurium, one of a new series of neuromuscular blocking drugs (Stenlake, 1979), has been reported to have a minimal effect on sympathetic mechanisms (Hughes and Chappie, 1981). However, the same authors suggest that, whilst indirect evidence indicated that histamine release was primarily responsible for the cardiovascular effects, a component of these effects may also be caused by sympathetic blockade.

It would therefore be instructive to examine the relative neuromuscular and ganglionic blocking potencies of atracurium and tubocurarine using isolated tissue preparations, from the same species, in which the effects of a range of drug concentrations may be compared.

METHOD

The effects of atracurium or tubocurarine on neuromuscular and ganglion transmission were examined using the guineapig isolated phrenic nerve—diaphragm preparation and the guineapig isolated hypogastric nerve—vas deferens preparation respectively.

Guineapigs were killed by a blow on the head and bled from an incision in the throat.

The guineapig isolated phrenic nerve—diaphragm preparation

The left phrenic nerve and hemidiaphragm were dissected from male or female guineapigs (600—800 g) as described for the rat by Bulbring (1946), and set up in an organ bath containing 50 ml of Krebs solution at 37°C bubbled with 95% O2 and 5% CO2. A supramaximal rectangular wave stimulus of 0.2 ms pulse duration was applied to the nerve at a frequency of 0.1 Hz. The muscle contractions were detected with an isometric transducer and amplified and recorded on a Grass polygraph (multichannel model 7D). The preparation was allowed a settling period of at least thirty minutes before addition of the drug under test. During this period the preparation was washed at three minute intervals. At the end of the settling period baseline responses were recorded for three minutes, then drug was added. A contact time of four minutes was allowed after which the preparation was washed at least four times in
drug-free Krebs solution, with three minute intervals between each wash, to re-establish the baseline response. The effects of eight drug concentrations were measured consecutively. Five guineapig preparations were used for each drug.

The guineapig isolated hypogastric nerve–vas deferens preparation

The left hypogastric nerve, hypogastric plexus and vas deferens were dissected from a male guineapig (600–800 g) (Hukovic, 1963) and set up in a 50 ml bath of Krebs solution at 37 °C bubbled with 95% O₂ and 5% CO₂. Nerve and muscle were stimulated alternately using conventional bipolar platinum wire electrodes for the hypogastric nerve and parallel platinum wire electrodes for the post-ganglionic intramural nerve fibres in the vas deferens (Birmingham and Wilson, 1963). Supraximal rectangular wave stimuli were used (hypogastric nerve 40 V, intramural nerve fibres 110 V) of 0.2 ms pulse duration at a frequency of 20 Hz. Each stimulus was applied for 20 s, the time interval between stimuli being 2 min. Contraction of the vas (longitudinal shortening) was recorded using an isotonic transducer and Byrants chart recorder. The preparation was allowed a settling period of at least thirty minutes before addition of the first drug concentration. Drug was added immediately after a muscle contraction and allowed to act for a cycle of eight alternate nerve and muscle stimulations (total contact time 18 min 40 s). A cumulative dose–response curve was obtained by repeatedly doubling the concentration for each successive cycle of stimulations. The drug concentration was increased until complete blockade of the response to transmural stimulation was obtained. The effect of guanethidine 4 × 10⁻⁴ mol litre⁻¹ on the response to post-ganglionic stimulation was then recorded. The bath fluid was then changed every three minutes until the contraction responses of the vas deferens to pre-ganglionic and post-ganglionic stimulation returned to their pre-drug level. Vasa deferentia from five guineapigs were used.

DRUGS

The responses of the guineapig phrenic nerve – hemidiaphragm and the hypogastric nerve – vas deferens preparations were examined using either atracurium (10⁻⁷ M – 1.28 × 10⁻⁵ M and 10⁻⁶ M – 5.12 × 10⁻⁶ M respectively) or tubocurarine (10⁻⁷ M – 2.6 × 10⁻⁶ M and 10⁻⁶ M – 1.28 × 10⁻⁵ M respectively). These drug concentrations are expressed as moles of drug per litre of bath fluid. Assuming a total e.c.f. volume of 9 litre for a 60 kg man, 10⁻⁵ mol litre⁻¹ (12 µg ml⁻¹ atracurium, 0.7 µg ml⁻¹ tubocurarine) equates to a dosage of 1.86 mg kg⁻¹ (atracurium) and 1.04 mg kg⁻¹ (tubocurarine).

A 10⁻² M stock solution of the dibesylate salt of atracurium was made in Krebs solution just prior to use and was acidified by the presence of 10⁻² M ascorbic acid to avoid decomposition via the Hoffman route (Hughes and Chappie, 1981). In the bath fluid ascorbate was thus present in equimolar concentrations to the drug, and at those levels did not alter the physiological pH of the gassed Krebs solution. Guanethidine and tubocurarine were similarly treated (Drury, Birmingham and Healy, 1980). The composition of the Krebs solution is as follows (mmol litre⁻¹): NaCl 119, KCl 4.7, MgSO₄.1.2, NaH₂PO₄ 0.9, CaCl₂ 2.5 and glucose 11.1.

RESULTS

Both atracurium and tubocurarine reduce the contraction response of the diaphragm and the vas deferens to phrenic nerve and hypogastric nerve stimulation respectively. An example of the recordings obtained are shown in figures 1 and 2. The mean values for the contraction responses expressed as a percentage of the control values were plotted as a log concentration response curve shown in fig. 3. For each preparation regression analysis of the linear part of the curve allowed determination of the concentrations of drug which would produce a 50% blockade (EC₅₀). These values, together with the Pearson’s product Moment Correlation Coefficients R, are shown in table I. The coefficient R defines the degree of relation between the two variables log dose and response. A value of 0 indicates no systematic relation whilst a value of ± 1 indicates a perfect correlation. Using statistical tables for the distribution of R the results obtained were shown to lie within 99% confidence limits (P = 0.01).

Atracurium did not reduce the response to transmural (post-ganglionic) stimulation but in fact slightly enhanced it. The mean and SEM inhibition of the response to transmural stimulation was −2.9% ± 0.61.

Guanethidine (4 × 10⁻⁴ mol litre⁻¹) completely blocked the response of the vas deferens to transmural stimulation. However this blockade was reversed by changing the stimulation rate from 20 Hz to 0.2 m⁻¹ (fast) to 1 Hz 100 m⁻¹ (slow).

The separation between the neuromuscular and ganglionic blocking potencies of the two drugs was
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Fig. 1. The effect of increasing concentrations of atracurium on the contraction response of the vas deferens to preganglionic, hypogastric nerve, stimulation (N1, N2, N3, N4, etc.) and to post-ganglionic, intramural nerves, stimulation (M1, M2, M3, etc.).

Fig. 2. The contraction response of the diaphragm to phrenic nerve stimulation and the blocking action of atracurium $3.2 \times 10^{-6}$ mol litre$^{-1}$ on the contraction response. The effect of three periods, two of 6 min and one of 12 min, of washout of drug are shown.

Fig. 3. Log dose–response curves for atracurium and for tubocurarine on the response of the diaphragm to phrenic nerve stimulation (---) and the response of the vas deferens to hypogastric nerve stimulation (-----). The mean and standard error of the response for each concentration of drug tested are shown ($n = 5$).
determined from the ratio between the EC50 values for ganglionic and neuromuscular blockade (equipotent molar ratio, EPMR). This separation was shown to be greater for atracurium than for tubocurarine, the values for EPMR being 48 and 9.4 respectively, see table I.

Table I. Mean concentration (with 99% confidence limits) for 50% reduction of the response (EC50) of the hemidiaphragm (NMB) and the vas deferens (GB). EPMR = Equipotent Molar Ratio i.e. (EC50 GB/EC50 NMB). R = Pearson's Product Moment Coefficient

<table>
<thead>
<tr>
<th>Drug</th>
<th>NMB (mol litre⁻¹)</th>
<th>GB (mol litre⁻¹)</th>
<th>EPMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atracurium</td>
<td>1.28 x 10⁻⁶</td>
<td>6.15 x 10⁻⁵</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>R = 0.967978</td>
<td>R = 0.821636</td>
<td></td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>3.44 x 10⁻⁶</td>
<td>3.24 x 10⁻⁵</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>R = 0.938087</td>
<td>R = 0.919568</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The separation between neuromuscular and sympathetic ganglionic blockade is an important consideration in the assessment of the clinical value of a neuromuscular blocking drug and has been examined using in vivo techniques (Hughes and Chappie, 1976). However other factors such as histamine release, vagal blockade or compensatory mechanisms may disguise the magnitude of the sympathetic ganglionic blockade. These effects may be avoided by the use of isolated tissue preparations (Birmingham and Hussain, 1980). In the present study the separation between neuromuscular and ganglionic blockade has been examined using isolated tissues from the same species. The neurogenic identity of the post-ganglionic stimulation was validated by observing the reversal of the blockade induced by guanethidine by changing the transmural stimulus parameters (Bentley and Sabine, 1963; Birmingham and Wilson, 1963). The muscle does not respond to direct stimulation at high frequencies, and therefore contraction responses are associated with post-ganglionic nerve stimulation. However, guanethidine blocks the neuromuscular junction on the vas deferens and hence the response to nerve stimulation. The muscle will nonetheless contract if stimulated directly. This, however, requires the use of slower frequencies of stimulation.

Results of the EC50 values for tubocurarine obtained in the present study are comparable to those obtained by Birmingham and Hussain (1980). For neuromuscular blockade EC50 values (10⁻⁶ mol litre⁻¹) were 3.44 and 6.7; for ganglion blockade they were 32.4 and 45 (present study quoted first).

Comparison of the EPMR values obtained in the present study and those reported for other muscle relaxants by Birmingham and Hussain (1980) suggest that, in the separation of neuromuscular and ganglionic blockade, atracurium is second only to pancuronium and five times that of tubocurarine. The EPMR values are as follows: atracurium 48 (present study); tubocurarine 9.4 (present study); tubocurarine 6.7; pancuronium 200; gallamine 24.3; alcuronium 22.9 and fazandinium 2.3 (Birmingham and Hussain, 1980).

Hughes and Chapple (1981) used in vivo studies to assess the degree of separation between neuromuscular, sympathetic and vagal blockade. Their results suggested that atracurium is unlikely to have more than a negligible effect on ganglionic transmission at concentrations required to produce a neuromuscular block. Their EPMR values for neuromuscular/vagal blockade were: atracurium 24.4, tubocurarine 1.5.

The effect of atracurium on the sympathetic system was also examined by Hughes and Chapple (1981) using in vivo techniques. In experiments performed using cats and monkeys they found that significant hypotension was evident at a dose 16 times greater than that required for full neuromuscular blockade (4 mg kg⁻¹), although in dogs 2 mg kg⁻¹ reduced mean arterial pressure to 53 ± 20.2% of the control value, and this latter finding agrees closely with the results presented here. Nonetheless, it would appear that a high degree of separation between the dose of atracurium required to produce neuromuscular blockade and the dose required to produce sympathetic ganglion blockade is suggested with in vivo and in vitro studies.

Although it must be remembered that results, obtained by the use of in vivo and in vitro animal studies, may not be identical with those obtained in clinical practice, in vitro studies allow the study of drugs under standard conditions and enable a prediction of their relative effects at separate sites. From these results it is suggested that the use of atracurium is much less likely than tubocurarine to be associated with sympathetic blockade.
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REFERENCES


IN VITRO VERGLEICH ZWISCHEN NEUROMUSKULÄRER UND GANGLIENBLOCKENDER POTENZ VON ATRAKURIUM UND TUBOCURARIN

ZUSAMMENFASSUNG


COMPARACIÓN IN VITRO ENTRE LAS RELACIONES DE POTENCIA BLOQUEADORA NEUROMUSCULAR Y GANGLIONAL DEL ATRACURIO Y DE LA TUBOCURARINA

SUMARIO

Se usaron los resultados de experimentos in vitro en base a la preparación de vas deferens – nervio hipogástrico y de la preparación hemidiaphragma – nervio frénico de cochinillos de la India para determinar la separación entre los efectos bloqueadores ganglionares y neuromusculares del atracurio y de la tubocurarina. Se emplearon las líneas de regresión para calcular las concentraciones de cada substancia (límites de confianza del 99%) que pudieran provocar un bloqueo al 50% (EC50) de la transmisión ganglionar y neuromuscular. La relación molar equipotente, al usar valores EC50, en lo que se refiere al bloqueo ganglionar/neuromuscular fue del 48 para el atracurio y del 9,4 para la tubocurarina.