TOBRAMYCIN AND NEUROMUSCULAR TRANSMISSION IN THE RAT ISOLATED PHRENIC NERVE-DIAPHRAGM PREPARATION

M. DE ROSAYRO AND T. E. J. HEALY

SUMMARY

The effects of different concentrations of tobramycin, streptomycin, neomycin and gentamicin on the rat isolated phrenic nerve-diaphragm preparation are reported. Streptomycin, neomycin and gentamicin produced dose-dependent neuromuscular blockade. Tobramycin increased the muscle response at high concentrations (6.4 x 10^{-4} - 2.6 x 10^{-2} mol litre^{-1}), but had no detectable effect when used in therapeutically recommended concentrations.

Several aminoglycoside antibiotics, such as streptomycin, kanamycin and neomycin, produce neuromuscular blockade in animals (Brazil and Corrado, 1957; Corrado and Ramos, 1958; Pittinger and Long, 1958; Timmerman, Long and Pittenger, 1959) and in man (Weber, 1957; Loder and Walker, 1959; Mann and Levin, 1960; Bush, 1961). There is clinical (Warner and Sanders, 1971; Hall et al., 1972) and experimental evidence (Brazil and Prado-Franceschi, 1969; Paradeli et al., 1974) that gentamicin, in large doses, can produce neuromuscular blockade. However, using lower, therapeutically recommended concentrations of gentamicin on the rat isolated phrenic nerve-diaphragm preparation, Drury and Healy (1975) were unable to demonstrate an effect on neuromuscular transmission. Tobramycin, a recently introduced aminoglycoside antibiotic, has an in vitro spectrum for anti-bacterial activity similar to that of gentamicin (Del Bene and Farrar, 1972) and appears to have a similar degree of toxicity (Welles et al., 1973). Thus tobramycin may be expected also to decrease neuromuscular transmission. The effects of varying concentrations of tobramycin on neuromuscular transmission were assessed using the rat isolated phrenic nerve-diaphragm preparation.

METHODS

The left phrenic nerve and a wedge of diaphragm of adult, male Wistar rats (weight 250-350 g) were dissected (Bulbring, 1946) and mounted in a water-bath filled with Krebs' solution at 37 °C. Oxygen 95% and carbon dioxide 5% were bubbled through the Krebs' solution.

A supramaximal rectangular wave stimulus (0.5-s pulse duration) was applied to the phrenic nerve six times per minute. Isometric tension developed by the muscle was recorded using a force transducer (Scientific Research Instrumentation) and a pen recorder (Servoscribe) or an ultraviolet recorder (S.E. Laboratories). Drugs were added at 15-min intervals for a 3-min contact time which was ended by washing four times with Krebs' solution during 12 min. Tubocurarine was added to the preparation in a molar concentration range of 3.2 x 10^{-7} to 2.0 x 10^{-5}, and the concentration required to produce an approximately 33% reduction in response was identified. Each of seven investigations was carried out using the following combinations and concentrations of drugs; five rats were used for each investigation.

1. Neomycin only: 6.4 x 10^{-4} to 2.6 x 10^{-3} mol litre^{-1}.
2. Streptomycin only: 6.4 x 10^{-4} to 2.6 x 10^{-3} mol litre^{-1}.
3. Gentamicin only: 6.4 x 10^{-4} to 2.6 x 10^{-3} mol litre^{-1}.
4. Tobramycin only: 6.4 x 10^{-4} to 2.6 x 10^{-3} mol litre^{-1} (1 mg ml^{-1}).
5. Tobramycin only: 5.0 x 10^{-9} (0.0023 μg ml^{-1}) to 1.6 x 10^{-4} mol litre^{-1} (74.7 μg ml^{-1}).
6. Tobramycin: 5.0 x 10^{-9} to 1.6 x 10^{-4} mol litre^{-1} plus tubocurarine 2.6 x 10^{-6} mol litre^{-1}.
7. Tobramycin: 5.0 x 10^{-9} to 1.6 x 10^{-4} mol litre^{-1} plus neostigmine 6.4 x 10^{-7} mol litre^{-1}.

RESULTS

The effects of high concentrations (6.4 x 10^{-4} - 2.6 x 10^{-3} mol litre^{-1}) of streptomycin, neomycin, gentamicin and tobramycin on the rat isolated phrenic nerve-diaphragm are shown in table I. Gentamicin, streptomycin and neomycin produced concentration-dependent decreases in contraction. At the maximum
TABLE I. Mean % change in contraction response and SEM (five observations). + = increase in response, - = decrease in response

<table>
<thead>
<tr>
<th>Molar concentration of drug</th>
<th>6.4 x 10^{-4}</th>
<th>1.3 x 10^{-3}</th>
<th>2.6 x 10^{-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>-2.2 ± 0.34</td>
<td>-10.4 ± 1.54</td>
<td>-47.3 ± 3.70</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>-4.4 ± 0.37</td>
<td>-19.2 ± 1.45</td>
<td>-61.1 ± 2.68</td>
</tr>
<tr>
<td>Neomycin</td>
<td>-2.8 ± 1.16</td>
<td>-19.3 ± 1.28</td>
<td>-88.8 ± 2.85</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>+4.9 ± 1.54</td>
<td>+9.6 ± 1.90</td>
<td>+13.9 ± 0.99</td>
</tr>
</tbody>
</table>

concentration used (2.6 x 10^{-3} mol litre^{-1}) the decreases were 47.3%, 61.1% and 88.8% respectively. Tobramycin, on the other hand, did not decrease the response at any concentration but produced small concentration-dependent increases in contraction which at the highest concentration (2.6 x 10^{-3} mol litre^{-1}) amounted to a mean potentiation of 13.9% (P < 0.001).

The effects of lower concentrations of tobramycin (5 x 10^{-9}-1.6 x 10^{-4} mol litre^{-1}) are shown in table II. At low concentrations, tobramycin produced small, non-significant changes in response. Tubocurarine in a concentration of 2.6 x 10^{-6} mol litre^{-1} produced a mean decrease in response of 35.8% (SEM 2.08) (five preparations) and this effect was not altered by tobramycin in concentrations up to 1.6 x 10^{-4} mol litre^{-1}. Neostigmine, when used alone in a concentration of 6.4 x 10^{-7} mol litre^{-1}, increased the contraction response by a mean of 19.36% (SEM 2.2) (five preparations). No change in this effect was detected after the addition of tobramycin in concentrations up to 1.6 x 10^{-4} mol litre^{-1}.

DISCUSSION

We have shown that, using the rat isolated phrenic nerve–diaphragm preparation, neomycin, streptomycin and gentamicin in high concentrations produce neuromuscular blockade and this confirms previous work using the same preparation (Brazil and Prado-Franceschi, 1969; Paradellis et al., 1974). However, the clinical importance of any neuromuscular blockade produced by an antibiotic must be related to the concentration required for effective antibacterial action.

Streptomycin and neomycin have been shown to produce neuromuscular blockade in clinical practice (Weber, 1957; Loder and Walker, 1959; Mann and Levin, 1960; Bush, 1961) and although Drury and Healy (1975), using the isolated phrenic nerve–diaphragm preparation, detected no change in neuromuscular transmission when gentamicin was used in small, yet clinically effective, concentrations, there have been case reports of neuromuscular blockade in clinical practice associated with gentamicin therapy (Warner and Sanders, 1971; Hall et al., 1972). It is true that results obtained using an isolated experimental preparation may not be identical with those observed in clinical practice. However, an isolated preparation allows the effect of different drugs to be studied under standard experimental conditions, and an indication of their relative effect may be obtained. In the present study, different aminoglycoside antibiotics were compared under the same conditions and dose ranges. It was found that tobramycin increased the muscle response in high concentrations but had no effect when used in therapeutically recommended concentrations. Furthermore, in concentrations up to 74.7 µg ml^{-1}, six times the toxic concentration (Stratford and Dixon, 1974), tobramycin did not alter detectably a partial blockade induced with tubocurarine nor the augmented response produced by neostigmine. In a toxicological study, Welles and others (1973) injected tobramycin and gentamicin i.v.

TABLE II. Mean % change in contraction response and SEM for five observations. (+ = increase in response, - = decrease in response)

<table>
<thead>
<tr>
<th>Molar concentration of drug</th>
<th>2.6 x 10^{-6}</th>
<th>2.0 x 10^{-5}</th>
<th>1.6 x 10^{-4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>+0.8 ± 0.97</td>
<td>+0.9 ± 0.81</td>
<td>+2.3 ± 0.69</td>
</tr>
<tr>
<td>Tobramycin + neostigmine</td>
<td>+18.86 ± 6.34</td>
<td>+26.64 ± 8.55</td>
<td>+21.78 ± 4.19</td>
</tr>
<tr>
<td>6.4 x 10^{-7} mol litre^{-1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin + tubocurarine</td>
<td>-34.29 ± 2.62</td>
<td>-33.98 ± 2.2</td>
<td>-35.85 ± 2.08</td>
</tr>
<tr>
<td>2.6 x 10^{-4} mol litre^{-1}</td>
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and using the cat soleus muscle preparation demonstrated neuromuscular blockade. However, an extremely high drug dose was used. In their study the maximum effect with tobramycin was produced within a dose range of 60–100 mg kg⁻¹ whereas comparable maximal effects were produced by 50 mg kg⁻¹ of gentamicin and 30 mg kg⁻¹ of neomycin. This evidence (Welles et al., 1973) suggests that tobramycin is less potent than neomycin or gentamicin in producing neuromuscular blockade.

The increase in contraction associated with the high concentrations of tobramycin (up to 1.2 mg ml⁻¹) noted in the present study has been observed with other antibiotics such as lincomycin (Sokoll, Cronnelly and Gergis, 1975), streptomycin (Dretchen et al., 1973) and clindamycin (Becker and Miller, 1976). Using lower concentrations of lincomycin (0.01–0.1 μg ml⁻¹), Sokoll, Cronnelly and Gergis (1975) reported an increase in miniature end-plate potential frequency, but at higher concentrations (10–100 mg ml⁻¹) the amplitude of the miniature end-plate potentials decreased and at the same time there was a decrease in end-plate sensitivity to iontophoretically applied acetylcholine. From these results it was suggested (Sokoll, Cronnelly and Gergis, 1975) that, in low concentrations, lincomycin increases the release of acetylcholine, whereas high concentrations decrease the receptor sensitivity. It may be that a similar mechanism is responsible for the concentration-dependent potentiation of muscle contraction by tobramycin reported in the present study.

Ootoxicity associated with the use of aminoglycoside antibiotics has been reported at concentrations greater than 10 μg ml⁻¹ (Wilson and Ramsden, 1977), whereas the bacterial minimum inhibitory concentration (MIC) is generally taken to be between 2 and 10 μg ml⁻¹ (Schoutens and Yourassowsky, 1973). The low tobramycin concentration range used in the present study (maximum concentration 74.7 μg ml⁻¹), which neither produced any detectable change in neuromuscular transmission nor influenced the effect of tubocurarine or neostigmine, exceeded greatly the ootoxic concentrations and the MIC. Therefore it appears that tobramycin is unlikely to produce neuromuscular blockade when used in effective antibacterial concentrations, and this may be a relevant factor in the choice of an aminoglycoside antibiotic for use in combination with muscle relaxants, in hypocalcaemia and in renal failure when accumulation of an aminoglycoside antibiotic may occur.

ACKNOWLEDGEMENT

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REFERENCES


**TOBRAMYCIN UND DIE NEUROMUSKULÄRE ÜBERTRAGUNG IN EINEM AUS EINER RATTE ISOLIERTEM PRÄPARAT AUS PHRENIKUSNERV UND ZWERCHFELL**

ZUSAMMENFASSUNG
Die Wirkungen verschiedener Konzentrationen von Tobramycin, Streptomycin, Neomycin und Gentamycin auf Rattenpräparate aus Phrenikusnerv und Zwerchfell werden beschrieben. Streptomycin, Neomycin und Gentamycin riefen eine dosisabhängige neuromuskuläre Blockierung hervor. Tobramycin erhöhte in hohen Dosen (6,4 x 10^{-4} bis 2,6 x 10^{-3} mol Liter^{-1}) die Muskelreaktion, hatte jedoch bei Verwendung in therapeutisch empfohlenen Dosen keine erkennbare Wirkung.

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**TOBRAMYCINE ET TRANSMISSION NEUROMUSCULAIRE DANS UNE PREPARATION DE NERF PHRENIQUE-DIAPHRAGME PRELEVES SUR UN RAT**

RESUME
On signale dans cet article les effets de différentes concentrations de tobramycine, streptomycine, némomycine et gentamicine sur une préparation de nerf phrénique-diaphragme prélevés sur un rat. La streptomycine, la némomycine et la gentamicine ont provoqué un blocage neuromusculaire qui a été fonction de la dose. La tobramycine à fortes concentrations a augmenté la réaction du muscle (6,4 x 10^{-4} à 2,6 x 10^{-3} mol litre^{-1}) mais n'a eu aucun effet décelable lorsqu'elle a été utilisée dans des concentrations thérapeutiques recommandées.

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**TOBRAMICINA Y TRANSMISION NEUROMUSCULAR EN LA PREPARACION AISLADA DE NERVIO FRENICO-DIAFRAGMA DE LA RATA**

SUMARIO
Se informa sobre los efectos de diversas concentraciones de tobramicina, streptomicina, neomicina y gentamicina sobre la preparación aislada de nervio frénico-diafragma de la rata. La streptomicina, neomicina y gentamicina produjeron un bloqueo neuromuscular dependiente de la dosis. La tobramicina aumentó la respuesta muscular en elevadas concentraciones (6,4 x 10^{-4} a 2,6 x 10^{-3} mol litro^{-1}) pero no ejerció efecto notable alguno cuando se usó en concentraciones terapéuticamente recomendadas.