INTERACTIONS OF DIISOPROPYL PHENOL (ICI 35 868) WITH SUXAMETHONIUM, VECURONIUM AND PANCURONIUM IN VITRO

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SUMMARY

In vitro studies were performed using the rat phrenic nerve–hemidiaphragm preparation to investigate possible interactions between diisopropyl phenol and its solvent, cremophor, with three neuromuscular blocking drugs. Cumulative concentration curves were constructed for the neuromuscular blockers and linear regression analyses performed. Differences in the calculated effective concentration to produce a 50% decrease in twitch height (EC50) and slope showed that diisopropyl phenol potentiated the action of suxamethonium, vecuronium (Org NC45) and pancuronium. Cremophor potentiated the action of suxamethonium but antagonized the action of the non-depolarizing neuromuscular blockers. The possible mechanisms of action are discussed.

Diisopropyl phenol has been investigated as an induction agent for general anaesthesia and may be administered by either intermittent bolus or continuous infusion to maintain unconsciousness (Briggs et al., 1981; Major et al., 1981; Verniquet et al., 1981). Since interactions have been reported between neuromuscular blocking drugs and other i.v. hypnotics (Booij and Crul, 1979; Krieg et al., 1980), it seemed appropriate to assess the likelihood of any interaction between diisopropyl phenol and certain neuromuscular blocking drugs.

A study was undertaken to look for possible interactions between diisopropyl phenol (or cremophor, the solvent for diisopropyl phenol) and suxamethonium, vecuronium and pancuronium using the rat phrenic nerve–hemidiaphragm preparation, and to detect the site of action if interaction did occur.

MATERIALS AND METHODS

Isolated rat phrenic nerve–hemidiaphragm preparations (Bülbbring, 1946) were obtained from Sprague–Dawley rats of 250–300 g body weight. The preparations were placed in double-walled baths, perfused with mammalian Kreb’s solution (sodium chloride 113.0; potassium chloride 4.7; calcium chloride 2.5; magnesium sulphate 1.2; sodium bicarbonate 25.0; sodium dihydrogen phosphate 2.5; and glucose 11.5 mmol litre⁻¹), aerated with 5% carbon dioxide in oxygen. Temperature (37°C) and pH (between 7.35 and 7.45 unit) were maintained constant. The techniques of stimulation and of recording the twitch response were those described by Foldes and colleagues (1969), using supramaximal twitch stimuli of 0.2 ms at 0.1 Hz to the phrenic nerve. When the twitch heights had remained steady for at least 20 min, either 1% diisopropyl in 16% cremophor, 30 µg ml⁻¹, or 16% cremophor alone were added to two-thirds of the baths. One-third of the baths had no drugs added and were used as controls. The concentration of cremophor when added alone was the same as that used as the solvent for diisopropyl and neither the diisopropyl phenol (plus cremophor) or the cremophor alone caused depression of twitch height when added to the bath. After another 30 min at the new steady state, cumulative concentration–response curves were determined using eight preparations for pancuronium, vecuronium (Org NC45) and suxamethonium with each bath solution (blank, diisopropyl phenol, or cremophor alone). Linear regression analyses were used to calculate the lines of best fit. At the end of each experiment, the preparation was washed with fresh Kreb’s solution and the recovery observed.

Finally, in 10 preparations the neuromuscular junction was blocked with a large dose of pancuronium. Diisopropyl phenol was added to five of these diaphragm preparations to a concentration of...
10 μg ml⁻¹. Every 20 min the concentration of diisopropyl phenol was increased by 10 μg ml⁻¹ while the muscle of the diaphragm was stimulated directly at 0.1 Hz. The other five preparations received no diisopropyl, were stimulated similarly, and served as controls. Cumulative concentration–response curves for diisopropyl phenol were constructed and fitted by linear regression analysis.

Comparisons of the EC₅₀ and slope of the dose–response curve of each neuromuscular blocker in the different bath solutions were made by analysis of variance and Student’s t test for unpaired data (Cooper, 1969). The same method was used to compare the directly stimulated diaphragms with either diisopropyl or nothing added to the bath. P<0.05 was considered significant.

RESULTS

The cumulative concentration–response curves for suxamethonium, vecuronium and pancuronium (fig. 1) show that diisopropyl phenol shifted the curves of all three blocking drugs to the left. The greatest effect occurred with suxamethonium. Cremophor alone caused a shift in the curve for suxamethonium to the left and the curves for the non-depolarizing blockers to the right. Washing the preparation at the end of each experiment did not result in complete recovery of twitch height.

The mean concentrations ± the standard deviation required to produce a 50% twitch depression (EC₅₀) and the slope of the calculated concentration–response curve are shown in table I. For suxamethonium, the EC₅₀ and slope of each combination were different from every other combination (P<0.01). For vecuronium, the EC₅₀ of each combination was also different from every other combination (P<0.01). The slope of the combination of vecuronium + blank curve was similar to the slope of the vecuronium + cremophor curve, but the slope of the vecuronium + diisopropyl phenol curve was different from the other two combinations (P<0.01). The EC₅₀ and slope of the pancuronium + cremophor curve was similar to that produced by pancuronium + blank. However, they were both different (P<0.01) when comparisons were made between pancuronium + diisopropyl and the other two combinations.

At the greatest concentration of diisopropyl tested, 40 μg ml⁻¹, significant blockade of the directly stimulated diaphragms occurred when diisopropyl was added to the bath. A 70% depression of twitch height occurred, compared with a 17% depression of twitch height in the control preparations (mean values, P<0.01).

DISCUSSION

Diisopropyl phenol in the rat phrenic nerve–hemidiaphragm preparation potentiated the action of all three neuromuscular blocking drugs studied. Possible mechanisms of action would include a direct effect on the postjunctional membrane receptor, direct effect on the muscle, inhibition of acetylcholine release, and interference with cholinesterase activity. Since we could not obtain complete recovery upon washing the preparation, and because the response to direct stimulation of the muscle was inhibited by diisopropyl, it appears that...
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TABLE I. Concentrations at which 50% depression of the twitch height occurred (EC₅₀) and slopes of the concentration–response curves for suxamethonium, vecuronium and pancuronium in the rat phrenic nerve–hemidiaphragm preparation (n = 8 for each group). *P < 0.01 v. both other groups with the same blocking drug; †P < 0.01 v. the group with diisopropyl phenol and the same neuromuscular blocker. Other comparisons were not statistically significant.

<table>
<thead>
<tr>
<th>Drug groups</th>
<th>EC₅₀(μg ml⁻¹)</th>
<th>Slope</th>
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<tbody>
<tr>
<td>(1) Suxamethonium</td>
<td>5.13 ± 0.57*</td>
<td>1.47 ± 0.27*</td>
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<tr>
<td>(2) Suxamethonium + cremophor</td>
<td>3.81 ± 0.23*</td>
<td>1.24 ± 0.34*</td>
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<tr>
<td>(3) Suxamethonium + diisopropyl phenol</td>
<td>2.49 ± 0.23*</td>
<td>0.54 ± 0.12*</td>
</tr>
<tr>
<td>(4) Vecuronium</td>
<td>3.03 ± 0.12*</td>
<td>1.32 ± 0.12†</td>
</tr>
<tr>
<td>(5) Vecuronium + cremophor</td>
<td>4.17 ± 0.41*</td>
<td>1.19 ± 0.18†</td>
</tr>
<tr>
<td>(6) Vecuronium + diisopropyl phenol</td>
<td>1.77 ± 0.25*</td>
<td>0.88 ± 0.09*</td>
</tr>
<tr>
<td>(7) Pancuronium</td>
<td>1.79 ± 0.11†</td>
<td>0.64 ± 0.88</td>
</tr>
<tr>
<td>(8) Pancuronium + cremophor</td>
<td>2.09 ± 0.30†</td>
<td>0.77 ± 0.16†</td>
</tr>
<tr>
<td>(9) Pancuronium + diisopropyl phenol</td>
<td>1.24 ± 0.21*</td>
<td>0.59 ± 0.16†</td>
</tr>
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</table>

This drug had an effect on the muscle itself which may be intracellular. Cremophor, however, appears to inhibit cholinesterase activity because it potentiates the effect of suxamethonium and inhibited the effect of the non-depolarizing blockers. We could speculate that, if diisopropyl was dissolved in a solvent which had not this interaction with non-depolarizing neuromuscular blockers, it would have had a greater potentiating effect on the non-depolarizing blockers in this in vitro preparation. The exact mechanism of action on the muscle itself is beyond the scope of this study. It is generally agreed that when drugs possess the same mechanism of action, the dose–response curves will have the same slope. Differences between the slopes in the suxamethonium groups are explicable because cremophor was demonstrated to have an inhibitory effect on cholinesterase activity while diisopropyl phenol had a direct effect on the muscle. The differences between the slopes in the vecuronium group may be caused partly by the fact that vecuronium is influenced to some extent by acetylcholinesterase (F. F. Foldes, personal communication). Further experiments should clarify these points.

Cremophor is known to bind to proteins and biological membranes, and was shown to decrease the onset time of pancuronium administered with cremophor-containing anaesthetics (Gramstad, Lilleasen and Minsaas, 1981). However, the proposed mechanism of action of cremophor was interference with the intravascular binding sites for pancuronium, leaving more drug free for diffusion and as this could not happen in the in vitro experiments presented here, it cannot be the sole mechanism of action.

We suggest further in vivo studies in both animals and man should be performed to determine if these findings have clinical implications for the possible concomitant use of diisopropyl phenol and neuromuscular blocking drugs.

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REFERENCES


INTERACTIONS ENTRE LE DIISOPROPYLPHENOL (ICI 35 868) ET LE SUXAMETHONIUM, LE VECURONIUM ET LE PANCURONIUM IN VITRO

RESUME
Des études in vitro ont été faites utilisant une préparation nerf phrénique-hémiaphragme de rat, afin de rechercher les interactions possibles entre le diisopropylphénol et son solvant, le crémophore, et trois agents bloquant la transmission neuromusculaire. Des courbes de concentration cumulative ont été établies pour les myorélexants et une analyse de régression linéaire pratiquée. Les différences observées dans la concentration calculée efficace pour obtenir une diminution de 50% de la hauteur du twitch (EC50) et dans la pente ont montré que le diisopropylphénol potentialisait l'action du suxamethonium, du vecuronium (OrgNC45) et du pancuronium. Le crémophore potentialisait l'action du suxamethonium mais antagonisait l'action des curares non dépolarisants. Les différents mécanismes d'action possibles sont discutés.

INTERAKTIONEN VON DIISOPROPYL-PHENOL (ICI 35 868) MIT SUXAMETHONIUM, VECURONIUM UND PANCURONIUM IN VITRO

ZUSAMMENFASSUNG

INTERACIONES DEL FENOL DI-ISOPROPIL (ICI 35 868) CON EL SUXAMETONIO, EL VECURONIO Y EL PANCURONIO IN VITRO

SUMARIO
Se llevaron a cabo estudios in vitro con una preparación de nervio frénico-hemidiafragma de rata con el objeto de averiguar las interacciones entre el fenol di-isopropil y su solvente, el cremofor, con tres substancias de bloqueo neuromuscular. Se establecieron las curvas de concentración cumulativa de los tres bloqueadores neuromusculares y se llevó a cabo el análisis de la regresión lineal. Las diferencias en la concentración efectiva calculada para producir un descenso del 50% de la altura de contracción (EC50) y de la inclinación indicaron que el fenol di-isopropil hacía más potente la acción del suxametónio, del vecuronio (OrgNC45) y del pancuronio. El cremofor hacía más potente la acción del suxametónio pero antagonizaba la acción de los bloqueadores neuro-musculares no-dепolarizantes. Se discute de los posibles mecanismos de acción.