IN VITRO STUDY OF INTERACTIONS BETWEEN I.V. ANAESTHETICS AND NEUROMUSCULAR BLOCKING AGENTS

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SUMMARY

The interactions of four anaesthetic drugs (ketamine, propanidid, Althesin and methohexitone) with two neuromuscular blocking agents (suxamethonium and pancuronium) have been investigated. On the isolated rat phrenic nerve-diaphragm preparation, all the anaesthetic drugs examined potentiated suxamethonium more than they potentiated pancuronium. The anticholinesterase agent, ecolithiopate, had no significant effect on the potentiation of suxamethonium caused by the anaesthetic drugs. At low concentrations the anaesthetic drugs increased the twitch tension elicited by stimulation of the phrenic nerve, whilst at high concentrations this potentiation was followed by blockade of neuromuscular transmission. With the exception of Althesin, all the anaesthetics decreased the sensitivity of the frog rectus preparation to acetylcholine. The possible sites and mechanisms of these interactions are discussed.

Interactions between i.v. anaesthetic drugs and neuromuscular blocking agents are known clinically and are of importance in the management of anaesthesia. Ketamine has been reported to potentiate (Bovill et al., 1971) or to have no effect on suxamethonium blockade (Rossi and Paggiali, 1975). Ketamine has been reported to potentiate tubocurarine also (Cronelly et al., 1973; Johnston, Miller and Way, 1974).

It is accepted widely that propanidid potentiates suxamethonium in vivo (Monk and Norman, 1972) and in vitro (Ellis, 1968). However, its interactions with non-depolarizing neuromuscular blocking agents are uncertain.

Althesin has been found to have no significant effects on the actions of muscle relaxants (Carson, Clarke and Dundee, 1973), but this lack of activity has not been investigated fully in vitro.

The following experiments were designed to make an in vitro, quantitative assessment of the interactions between four i.v. anaesthetics (ketamine, propanidid, Althesin and methohexitone) and the neuromuscular blocking agents, suxamethonium and pancuronium, and to examine also possible mechanisms of these interactions.

Increased neuromuscular activity, such as increased tone, tremor and fasciculations, have been found to occur during the induction of anaesthesia with ketamine (Pender, 1971), propanidid, Althesin and methohexitone (Clarke et al., 1972). Although the effects of ketamine are thought to be the result of central stimulation (Johnston, Miller and Way, 1974), the role of peripheral mechanisms has not been excluded. Furthermore, it has been shown that the barbiturates (Sirnes, 1954) and propanidid (Ellis, 1971) act directly on striated muscle. Therefore, the direct actions of the anaesthetic drugs on the rat diaphragm preparation have been examined also.

METHODS

Rat phrenic nerve-diaphragm preparation

The rat phrenic nerve-diaphragm preparation (Bulbring, 1946) was set up under 4 g tension in an organ bath containing 75 ml of Krebs' solution maintained at 37 °C, and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The preparation was stimulated either indirectly via the phrenic nerve (0.1 ms duration, 0.1 Hz, supramaximal voltage) or directly via the muscle (5 ms duration, 0.1 Hz, supramaximal voltage). Isometric contractions were measured using an "Ether" dynamometer connected to a Grass 79 C Polygraph.

Various concentrations of the anaesthetic drugs were added to the bath and the effects noted. Then, using concentrations of the anaesthetic drugs which did not block neuromuscular transmission, their interactions with neuromuscular blocking agents were examined. Log concentration–effect curves for either suxamethonium or pancuronium were obtained using a 4-min contact time for each concentration and a dose cycle of 16 min. This was repeated in the...
presence of various concentrations of ketamine, propanidid, Althesin or methohexitone, which were added to the bath 15 min before the neuromuscular blocking agent. The ranges of concentrations used for each anaesthetic are shown in table I. Dose ratios were obtained for suxamethonium or pancuronium in the presence of the various concentrations of anaesthetic drugs. These were measured as the displacement of the suxamethonium or pancuronium concentration–effect curve along the x axis, that is the difference between the EC$_{50}$ values for the neuromuscular blocking agents in the presence and absence of anaesthetic agent.

The same procedures were performed in the presence of an irreversible anticholinesterase agent, ecatiopate iodide. The tissue was exposed to ecatiopate for 45 min before the addition of the neuromuscular blocking agent or anaesthetic drug. The concentration of ecatiopate (5.22 × 10$^{-6}$ mol/litre) was that which abolished completely the antagonism by neoestigmine 8.97 × 10$^{-6}$ mol/litre of the neuromuscular block produced by an EC$_{50}$ concentration of pancuronium 3 × 10$^{-6}$ mol/litre.

**Frog rectus abdominis preparation**

The isolated frog rectus abdominis preparation was set up under a resting tension of 2 g in a 20-ml bath containing frog Ringer solution, bubbled with oxygen, at room temperature. Isometric contractions were recorded using an “Ether” dynamometer connected to a Rikidenki pen recorder.

Concentration–effect curves to acetylcholine were obtained using a 45-s contact time and an 8-min dose cycle. Responses were expressed as a percentage of the maximum response to acetylcholine. Concentration–effect curves for acetylcholine were repeated in the presence of various concentrations of the anaesthetic agents (table I). Dose ratios for acetylcholine in the presence of the anaesthetic drugs were measured as before.

**Calculation of results**

The effects of the anaesthetic drugs upon the responses to suxamethonium, pancuronium and acetylcholine were compared by calculating their pP$_2$ values if potentiation occurred (Pleuvry and Hunter, 1968) or pA$_2$ values (Schild, 1947) if antagonism occurred. To obtain these values the log (dose ratio — 1) was plotted against the negative logarithm of the molar concentration of anaesthetic (Arunlakshana and Schild, 1959). In the concentration range used the points were linear and the best straight line was plotted using the method of least squares (Goldstein, 1971). pA$_2$ values or pP$_2$ values with their 95% confidence limits were obtained from the intercept of this line with the x axis. The slopes of the plots were measured also, since differences in slopes for different drugs could indicate differences in the mechanisms by which they exert their actions. For example, in the assessment of antagonism, Arunlakshana and Schild (1959) found that, according to the law of mass action, one of the criteria necessary for competitive antagonism was that the slope of the plot of log (dose ratio — 1) against the negative logarithm of the molar concentration of antagonist should not differ significantly from the value of 1.

**RESULTS**

**Rat phrenic nerve–diaphragm preparation**

In the concentration range shown in table I all the anaesthetic drugs increased the twitch tensions elicited by stimulation of the phrenic nerve (fig. 1). This augmentation of twitch tension was abolished by pretreatment of the preparation with ecatiopate. Higher concentrations of the anaesthetic drugs produced an initial increase in twitch tension followed by blockade. Concentrations of anaesthetic drugs which completely blocked the response of the preparation to nerve stimulation reduced also the response to direct stimulation of the muscle. However, these effects of the anaesthetic agents were completely reversible, control responses being regained 10–20 min after washing the preparation with Krebs’ solution.

In the fully curarized preparation (tubocurarine 1 × 10$^{-4}$ mol/litre) only ketamine and methohexitone enhanced the twitch tension produced by direct stimulation of the muscle.

The responses of the rat phrenic nerve–diaphragm preparation to repeat exposures to either suxamethonium or pancuronium, in the absence of anaesthetic agents, showed no significant changes over a period
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Fig. 1. The effects of i.v. anaesthetic drugs upon the twitch tension evoked by stimulation of the phrenic nerve supplying the isolated rat diaphragm preparation. Each point is the mean percentage increase from pre-drug tension ± SEM from 30–36 experiments. □, K = ketamine; ○, P = propanidid; ■, A = Althesin; ●, M = methohexitone.

of several hours. However, in general, the variability of the responses to pancuronium was greater than the variability of the responses to suxamethonium.

All four anaesthetic drugs potentiated suxamethonium in a concentration-dependent manner. The concentration–effect relationships for suxamethonium showed a parallel shift to the left. Pancuronium, however, was potentiated by only three of the anaesthetic drugs. Althesin had no effect.

The potentiating effect of the anaesthetic drugs on the two neuromuscular blocking agents are expressed as pP\textsubscript{2} values (table II). Suxamethonium was potentiated to a much greater extent than was pancuronium. This is illustrated by the fact that the pP\textsubscript{2} values for all anaesthetics with suxamethonium were significantly greater than those with pancuronium.

The plots of log (dose ratio — 1) against the negative logarithm of the molar concentration of anaesthetic were linear over the concentration ranges used. The slopes of these plots are shown also in table II. With the exception of Althesin the slopes of all the plots for all anaesthetic drugs with suxamethonium are not significantly different from 1. However, with pancuronium, only ketamine and methohexitone produced slopes not significantly different from 1.

Ecothiopate increased the sensitivity of all preparations to suxamethonium (P<0.05). However, the anaesthetic drugs still potentiated suxamethonium in a concentration-related manner, and the degree of the potentiation was not decreased by the presence of ecothiopate (table II). Indeed, the pP\textsubscript{2} value for ketamine in the presence of ecothiopate was greater than that obtained in its absence, suggesting that ketamine had more pronounced suxamethonium-potentiating properties in the presence of ecothiopate.

**Frog rectus abdominis preparation**

The contractions of the frog rectus to acetylcholine were reduced by three of the anaesthetic agents (ketamine, propanidid and methohexitone) and they caused a concentration-dependent, parallel shift to the right of the acetylcholine concentration–effect curve.

The Arunlakshana and Schild (1959) plots gave straight lines over the concentration range used. The pA\textsubscript{2} values and the slopes of the plots for the three anaesthetic agents are shown in table III. Only the slope for propanidid was close to 1.

In no instance did the high concentrations of ketamine and methohexitone cause any contraction of the frog rectus abdominis muscle. However, relatively high concentrations of propanidid and Althesin induced a slow contraction of the muscle, which developed over 20 min. It must be noted that these concentrations were considerably greater than those used earlier in the study.

The solution enhancing agent, used in the commercial preparations of Althesin, cremophor-ELB, neither caused any change in the twitch tension nor modified the actions of the neuromuscular blocking agents used in this study.

**DISCUSSION**

The present investigation has demonstrated the twitch-facilitating effects of four anaesthetic drugs on contractions of the rat phrenic nerve–diaphragm preparation stimulated via the phrenic nerve. Ketamine and methohexitone increased also twitch tension in directly stimulated fully curarized preparations, suggesting that these two anaesthetic agents
TABLE II. The potentiation of suxamethonium and pancuronium by four anaesthetic agents on the rat phrenic nerve-diaphragm preparation

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Suxamethonium</th>
<th>Ecothiopate + suxamethonium</th>
<th>Pancuronium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>3.59 (3.54–3.64)</td>
<td>4.10 (4.03–4.15)</td>
<td>3.26 (3.19–3.33)</td>
</tr>
<tr>
<td>Propanidid</td>
<td>1.31 (1.25–1.37)</td>
<td>1.43 (1.32–1.55)</td>
<td>0.61 (0.42–0.80)</td>
</tr>
<tr>
<td>Althesin</td>
<td>4.87 (4.83–4.91)</td>
<td>5.10 (4.80–5.40)</td>
<td>—</td>
</tr>
<tr>
<td>Methohexitone</td>
<td>5.24 (5.08–5.40)</td>
<td>5.29 (5.05–5.39)</td>
<td>4.71 (4.37–5.05)</td>
</tr>
</tbody>
</table>

The pP₂ values and their 95% confidence limits were obtained from the plots of log (dose ratio – 1) for the neuromuscular blocking agents against the negative logarithm of the concentration of anaesthetic.

The slopes refer to the regression lines corresponding to these plots. * Not significantly different from 1. The concentration of ecothiopate was 5.22 × 10⁻⁶ mol/litre.

TABLE III. Frog rectus abdominis preparation: the antagonism of acetylcholine by three anaesthetic agents

<table>
<thead>
<tr>
<th>Anaesthetic agent</th>
<th>pA₂ values</th>
<th>Slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>3.64 (3.49–3.8)</td>
<td>0.44</td>
</tr>
<tr>
<td>Propanidid</td>
<td>1.96 (1.87–2.05)</td>
<td>1.143</td>
</tr>
<tr>
<td>Methohexitone</td>
<td>5.53 (5.46–5.60)</td>
<td>1.285</td>
</tr>
</tbody>
</table>

pA₂ values and slopes were obtained from Arunlakshana and Schild (1959) plots (95% confidence limits are in parentheses).

have a postsynaptic site of action. This contrasts with the work of Bogdan, Glisson and El-Etr (1974) in the rabbit, where twitch-facilitating effects of ketamine and methohexitone were attributed to a presynaptic action on acetylcholine release. However, the findings in the present study are compatible with those of Hamilton and others (1972), who demonstrated the direct actions of ketamine upon rat muscle, and with the work of Sirnes (1954) who demonstrated similar actions of the barbiturates.

The increase in twitch tension produced by the anaesthetic drugs was abolished by pretreatment with the anticholinesterase agent, ecothiopate. Whilst this might suggest that the mechanism for twitch facilitation by the anaesthetics involved anticholinesterase activity, it could be explained also by the observation that anticholinesterase agents sensitized preparations to the depressant actions of the anaesthetic drugs (Sirnes, 1954).

In high concentrations ketamine, propanidid and methohexitone blocked neuromuscular transmission. This may be related to the reported local anaesthetic activity of these three anaesthetic agents (Quilliam, 1955; Dowdy, Kaya and Gocho, 1971; Ellis, 1971), as local anaesthetic drugs have been shown to block neuromuscular transmission by virtue of their membrane-stabilizing action (Deguchi and Narahashi, 1971).

The present investigation on the isolated rat phrenic nerve-diaphragm preparation shows clearly that the four anaesthetic agents potentiated the neuromuscular blocking activity of suxamethonium in vitro. Since suxamethonium is metabolized rapidly by pseudocholinesterase, inhibition of cholinesterase activity could be a mechanism by which anaesthetic agents potentiate suxamethonium. However, the potentiation of suxamethonium by all four anaesthetic drugs, as measured by the pP₂ values, was not decreased by pretreatment with the anticholinesterase agent, ecothiopate. Thus it is unlikely that anticholinesterase activity is involved in the potentiation of suxamethonium by the anaesthetics in vitro. This is confirmed by the data obtained using the frog rectus abdominis preparation in which the anaesthetic agents decreased the sensitivity to acetylcholine.

The above findings are supported by the work of
Schuh (1975) who found that interference with hydrolysis of suxamethonium by ketamine was negligible and could not account for the potentiation of suxamethonium reported in both man and animals. However, some authors have suggested that propanidid intensifies the actions of suxamethonium by interference with biotransformation (Torda, Burkhart and Toh, 1972) although Ellis (1968) could not demonstrate this in vitro and Doenice (1965) noted that the inhibition of cholinesterase by propanidid was extremely transient. In man, Althesin has no detectable cholinesterase-inhibiting activity (Wisborg, Hanel and Viby, 1974).

Whilst the present study has not been able to determine the exact mechanism by which certain anesthetic drugs potentiate neuromuscular blocking agents, it is clear that Althesin behaves differently from the other three. When potentiating suxamethonium, only Althesin was associated with a plot of log (dose ratio — 1) against the negative molar concentration of anaesthetic which was significantly different from 1. Furthermore, although the anesthetic agents were less effective as potentiators of pancuronium, only Althesin failed to have any effect at all. Finally, only Althesin failed to reduce the response to acetylcholine on the frog rectus preparation.

ACKNOWLEDGEMENTS

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REFERENCES


ETUDE IN VITRO DES INTERACTIONS ENTRE AGENTS ANESTHESIANTS INTRA VEINEUX ET AGENTS DE BLOCAGE NEUROMUSCULAIRE

RESUME

L'auteur a étudié les interactions de quatre produits anesthésiants (kétamine, propanidid, Althéside et méthohexitone) et de deux agents de blocage neuromusculaire (suxaméthonium et pancuronium). Tous les produits anesthésiants étudiés sur une préparation de nerf phrénique-diaphragme du rat ont davantage renforcé le suxaméthonium qu'ils n'ont renforcé le pancuronium. L'épothiopate, agent d'anticholinesthérase, n'a eu aucun effet
significatif sur le renforcement du suxaméthonium causé par les produits anesthésiants. A faibles concentrations, les produits anesthésiants ont augmenté la tension de crispation provoquée par la stimulation du nerf phrénique, alors qu’à fortes concentrations ce renforcement a été suivi par un blocage de la transmission neuromusculaire. A l’exception de l’Althesine tous les agents anesthésiants ont fait décroître la sensibilité de la préparation du muscle rectus de grenouille à l’acétylcholine. On discute dans cette communication des sites et des mécanismes possibles de ces interactions.

STUDIE IN VITRO DER WECHSELWIRKUNGEN ZWISCHEN I.V. GEGBEBNEN NARKOSEMITTELN UND NEUROMUSKULÄREN BLOCKIERUNGSMITTELN

ZUSAMMENFASSUNG


ESTUDIO IN VITRO DE LAS INTERACCIONES ENTRE ANESTÉSICOS I.V. Y LOS AGENTES PARA BLOQUEO NEUROMUSCULAR

SUMARIO

Se han investigado las interacciones de cuatro anestésicos (quetamina, propanidid, Althesin y metohexitona) con dos agentes para el bloqueo neuromuscular (suxametonio y pancuronio). En la preparación aislada nervio frénico-diafragma de rata, todos los anestésicos examinados potenciaron más al suxametonio que al pancuronio. El agente anticolinesterásico, ecotiopato, no ejerció efecto significante sobre la potencialización del suxametonio causada por los anestésicos. A bajas concentraciones los anestésicos aumentaron la tensión espasmodica obtenida mediante estimulación del nervio frénico, mientras que a altas concentraciones esta potencialización fue seguida de bloqueo de la transmisión neuromuscular. Con excepción de Althesin, todos los anestésicos disminuyeron la sensibilidad de la preparación rectus de rana a la acetilcolina. Se comentan los posibles sitios y mecanismos de estas interacciones.