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The neuromuscular blocking effect of trimetaphan alone and in combination with different non-depolarizing muscle relaxants in the rat*

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The effect of trimetaphan alone and in combination with pancuronium, tubocurarine or metocurine (dimethyl tubocurarine) has been examined on the rat phrenic nerve diaphragm preparation. Trimetaphan alone produced neuromuscular blockade in an all-or-none fashion once a concentration of between $2{\cdot}39 \times 10^{-4}$ and $3{\cdot}00 \times 10^{-4}$ M had been exceeded. Concentrations of trimetaphan below this threshold produced a dose-dependent potentiation of all three non-depolarizing relaxants studied. This potentiation was equal for tubocurarine and metocurine, but less for pancuronium.

The principal site of action of the non-depolarizing muscle relaxants is the acetylcholine receptors on the post-junctional membrane of the neuromuscular junction. The classical view envisages receptor occlusion by the relaxant molecules preventing transmitter access and resulting in neuromuscular blockade. The neuromuscular junction has however proved to be much more complicated than was originally believed, evidence so far supporting the presence of more than one subtype of acetylcholine receptor situated not only on the post-junctional membrane but also on the pre-junctional nerve ending (see Standaert 1985). Those receptors on the post-junctional membrane are the classic nicotinic neuromuscular receptors, while those on the pre-junctional region may have a greater similarity to the nicotinic acetylcholine receptors in autonomic ganglia (Bowman 1980). Each non-depolarizing relaxant then has its own activity profile on this whole integrated system and differences in the behaviour of the individual drugs have been attributed to the result of a different emphasis on the pre- and post-junctional receptors (Pollard & Jones 1983).

Ganglionic blocking agents are known to enhance a tubocurarine neuromuscular blockade both in-vivo (Wilson et al 1976) and in-vitro (Deacock & Davies 1958), but data concerning other relaxants are lacking. Furthermore, in view of the different activity profile of each relaxant, it might be expected that such an interaction would not be identical for every agent. This study was therefore designed to test this hypothesis in-vitro.

METHODS

Wistar rats, 250–350 g were killed by cervical dislocation and exsanguination and both hemidiaphragms dissected out together with their accompanying phrenic nerves. These were transferred to a dish containing oxygenated Krebs solution at room temperature (20 °C), trimmed, attached to carrier assemblies and placed in identical 50 mL organ baths containing Krebs-Henseleit solution (Na^+ 143, Cl^- 129, K^+ 5·9, Ca^{2+} 3·3, Mg^{2+} 1·2, HCO_3^- 25, SO_4^{2-} 1·2, $H_2PO_4^-$ 1·2, glucose 11·1) at 37 °C, through which was bubbled 5% carbon dioxide in oxygen. The hemidiaphragms were stimulated indirectly through the nerve at a frequency of 0·1 Hz using 0·2 ms supramaximal voltage square wave stimuli. Direct stimuli, using 2 ms supramaximal voltage square wave pulses were applied across the muscle at intervals. The resulting twitch was recorded using an isometric force transducer and displayed on a Grass 79C polygraph. The resting tension on the muscle was maintained at 4 g. Agents were each added to the bath in a small volume to obtain the desired final concentration. The technique of dose accumulation was used with a contact time of 20 min, by which time steady state had been reached. The twitch height depression was measured and expressed as a per cent reduction from the initial control value. The bathing fluid was regularly refreshed, and each preparation used for the determination of no more than two concentration-response relationships, separated by a wash cycle of

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40 min incorporating at least six changes of fresh bathing solution. Trimetaphan camsylate was chosen as the ganglion blocking agent and tubocurarine chloride, metocurine (dimethyltubocurarine) iodide and pancuronium bromide as the muscle relaxants.

In the first part of the experiment the neuromuscular blocking effect of trimetaphan alone was investigated on six different preparations by addition of increasing concentrations to the bath. Three concentrations, each of which alone was less than the minimum producing neuromuscular blockade, were then chosen.

In the second part of the experiment, the three muscle relaxants were taken and concentration-response relationships established to each, both alone and in the presence of each of the three chosen concentrations. A given preparation was only exposed to one relaxant but the trimetaphan concentrations were varied in a random manner. Each different combination of a relaxant and trimetaphan was determined on six preparations. Concentration-response relationships were then constructed for each set of data, using the technique of least squares regression on a minimum of three points between 15 and 85% twitch height inhibition. The log EC25, log EC50 and log EC75 were determined from each log concentration-response graph. The mean log EC25, mean log EC50 and mean log EC75 were calculated for each set of six preparations in each group and these means used in the construction of the Figs. The EC50 ratio was also calculated for each group as the EC50 in the presence of trimetaphan divided by the EC50 in the absence of it. This ratio allowed comparisons between changes in the EC50s, i.e. shift of the log concentration-response graph, to be readily seen. The slopes of the four lines for each relaxant were compared using an analysis of variance.

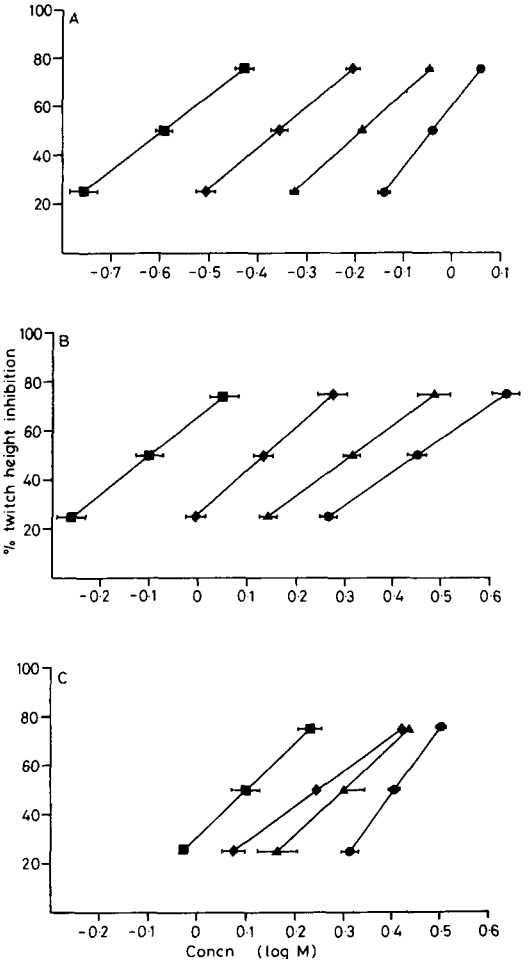

FIG. 1. Log concentration–response relationships to (A) tubocurarine, (B) metocurine and (C) pancuronium alone and in the presence of 3 increasing concentrations of trimetaphan (mean ± s.e.m.). Key: ● 0, ▲ $3{\cdot}35 \times 10^{-5}$, ◆ $1{\cdot}00 \times 10^{-4}$, ■ $1{\cdot}67 \times 10^{-4}$ M.

RESULTS

Trimetaphan alone

The results are shown in Table 1. In low concentrations, trimetaphan was without an effect. Once a threshold concentration of between $2{\cdot}39 \times 10^{-4}$ and $3{\cdot}00 \times 10^{-4}$ M had been exceeded, complete neuromuscular blockade resulted. The time taken to reach 100% inhibition of indirect twitch was inversely related to the trimetaphan concentration above this threshold level. The response to direct stimulation was unaffected by all concentrations used. The drug could be removed from the preparation by the wash cycle. There was no carry over, and repeating the same concentration produced an identical effect. From these results, trimetaphan $3{\cdot}35 \times 10^{-5}$, $1{\cdot}00 \times 10^{-4}$ and $1{\cdot}67 \times 10^{-4}$ M (below the threshold for 100% inhibition with trimetaphan alone) were chosen for the second part of the study.

Table 1. Neuromuscular blockade produced by trimetaphan. Concentrations from $3{\cdot}35 \times 10^{-5}$ to $2{\cdot}39 \times 10^{-4}$ M produced no twitch inhibition.

Trimetaphan concentration ($\times 10^{-4}$ M)	% Twitch inhib. (indirect stim.)	Time to 100% twitch inhib. (min) (mean ± s.e.m.)
3·00	100	77·3 ± 5·2
3·59	100	38·7 ± 2·5
4·19	100	23·3 ± 2·6
4·80	100	15·4 ± 1·1

Trimetaphan and non-depolarizing relaxants

The results for the three different relaxants are shown in Fig. 1. As the concentration of trimetaphan increased, there was a progressive shift to the left of the log concentration-response line. The spacing between the lines for tubocurarine and metocurine (Fig. 1A, B) was very similar, while the lines for pancuronium (Fig. 1C) were closer. The EC50 ratios (Table 2) display a measure of the degree of movement of the lines and confirm the previous observations. Those ratios for tubocurarine and metocurine were similar, showing an almost identical degree of shift, while those for pancuronium were considerably different, showing less shift of the pancuronium line by trimetaphan. Trimetaphan produced a change in the gradient of the log concentration-response lines, which was statistically significant for certain combinations, as indicated in Table 2.

Table 2. Changes in slope and EC50 of the log concentration-response graph for each relaxant in the presence of increasing concentrations of trimetaphan.

	Trimetaphan concn (M)			
	0	$3{\cdot}35 \times 10^{-5}$	$1{\cdot}00 \times 10^{-4}$	$1{\cdot}67 \times 10^{-4}$
Pancuronium				
Slope (mean ± s.e.m.)	2·80 ± 24	183 ± 9*	148 ± 10*	221 ± 25
EC50 (mean)	2·55	2·00	1·77	1·27
EC50 ratio	1·00	0·78	0·69	0·50
Tubocurarine				
Slope (mean ± s.e.m.)	262 ± 22	183 ± 5*	177 ± 13*	159 ± 9**
EC50 (mean)	0·91	0·65	0·44	0·26
EC50 ratio	1·00	0·71	0·48	0·29
Metocurine				
Slope (mean ± s.e.m.)	141 ± 12	161 ± 19	193 ± 17	160 ± 6
EC50 (mean)	2·82	2·06	1·35	0·79
EC50 ratio	1·00	0·73	0·48	0·28

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$. Slope in the presence of trimetaphan compared with that in its absence.

DISCUSSION

Trimetaphan has an inhibitory effect on indirectly, but not on directly, elicited muscle twitch, indicating that it exerts a blocking action at the neuromuscular junction. This is not a new finding, trimetaphan having previously been shown to have neuromuscular blocking properties in-vitro (Deacock & Davies 1958; Gergis et al 1977), in-vivo (Aguilar & Boldrey 1960; Deacock & Hargrove 1962) and also in the clinical situation (Payne 1957; Dale & Schroeder 1976; Wilson et al 1976; Nakamura et al 1980). We have observed what appears to be a threshold concentration below which there is no effect, and above which neuromuscular blockade results, the rate of block being dependent on the trimetaphan concentration. This 'all-or-none effect' has not previously been reported. Deacock & Davies (1958) showed that a concentration of $5{\cdot}01 \times 10^{-4}$ M produced 100% neuromuscular blockade, whereas $3{\cdot}34 \times 10^{-4}$ M did not. Although this may appear to represent an all-or-none effect, it was not recorded as such, and those authors did appear to leave the lower concentration in contact with the preparation for only approximately 4 min. Our observations would support the fact that in such a short time $3{\cdot}34 \times 10^{-4}$ M would not produce any apparent effect. Their other observation of 100% block with $5{\cdot}01 \times 10^{-4}$ M is entirely in agreement with our findings. Gergis et al (1977), using frog muscle at room temperature demonstrated a clear dose-related effect (not an all-or-none response) with trimetaphan concentrations of $1{\cdot}67 \times 10^{-5}$ to $1{\cdot}25 \times 10^{-4}$ M, which are about one-tenth of those used in our study.

The interactions between trimetaphan and the three non-depolarizing muscle relaxants shows it produced a dose-dependent potentiation of them but they did not all behave in the same way. Pancuronium is potentiated less than tubocurarine and metocurine, which are almost identical to each other. The actions of tubocurarine and metocurine are thought to be very similar and both differ from pancuronium (Lebowitz et al 1980).

Trimetaphan is a weak antagonist at the neuromuscular junction. When a second weaker competitive antagonist is added to a system already containing a competitive antagonist, then a reduction in the effect of the first antagonist would be expected, not potentiation (Ginsborg & Stephenson 1974). A simple interaction at one receptor site is therefore unlikely.

In conclusion, we have shown that trimetaphan potentiates individual non-depolarizing muscle relaxants differently in-vitro, although the exact sites of these interactions within the confines of the neuromuscular junction cannot be further elucidated from this study.

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