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A quantitative comparison of the antagonism of tubocurarine and diallylnortoxiferine by four anticurare agents

BARBARA J. PLEUVRY AND A. R. HUNTER

The antagonism of tubocurarine and diallylnortoxiferine by neostigmine, physostigmine, edrophonium and ambenonium, has been examined on the rat phrenic nerve diaphragm preparation. Ambenonium showed the greatest activity and physostigmine and edrophonium were the least potent and equally active. This order applied to both neuromuscular blocking agents, but all four antagonists show significantly greater activity against diallylnortoxiferine. The possibility that neostigmine and physostigmine have some qualitative differences in anticurare mechanism compared with edrophonium and ambenonium, on this preparation, is discussed.

THE anticurare actions of physostigmine, neostigmine (Bulbring & Chou, 1947), edrophonium (Randall, 1950) and ambenonium (Lands, Karczmar & others, 1955) are well known. However, their curare antagonism has been attributed to anticholinesterase activity alone (Hobbiger, 1952; Nastuk & Alexander, 1954), to presynaptic activity (Riker, Werner & others, 1959) and to a combination of these two mechanisms together with some direct activity on the motor end-plate (Blaber & Bowman, 1963).

If the four drugs mentioned antagonize tubocurarine by the same mechanism, they ought to show a similar relation between the concentration of antagonist and the degree of antagonism. The assessment of this relation was made [within the framework of the limitations set out by Rees (1966)] by the determination of pA_2 values (Schild, 1947) and by the application of a test for competitive antagonism (Arunlakshana & Schild, 1959).

There is some conflict in the literature about the ability of anticurare agents to antagonize a new neuromuscular blocking agent, diallylnortoxiferine (Hunter, 1964; Lund & Stovner, 1962; Foldes, Brown & others, 1963; Venn, 1965). Since no *in vitro* work on this subject has been published, the antagonistic potency of the four anticurare drugs against diallylnortoxiferine was also examined.

Experimental

METHODS

The rat phrenic nerve diaphragm preparation (Bulbring, 1946) and the apparatus described by Starmer & Thomas (1961) were used. The experimental procedure of Rees (1966) was followed except that both male and female rats, 200–300 g, were used and rectangular electric pulses of 0·8 msec duration were applied to the phrenic nerve at a frequency of 7/min.

From the Departments of Pharmacology and Anaesthesia, University of Manchester.

The neuromuscular blocking agents used were tubocurarine chloride and diallylnortoxiferine dichloride, and the antagonists were neostigmine methyl sulphate, physostigmine salicylate, ambenonium chloride and edrophonium chloride. All concentrations are expressed as final molar concentration in the bath.

The experiments were designed according to the method of Bulbring & Chou (1947). It was necessary, however, to extend the wash-out period, especially for ambenonium, to ensure adequate removal of the drug. Repeated additions of tubocurarine to a preparation, after a 15 min wash-out period, gave quantitatively similar responses, but such a preparation was found to be unexpectedly sensitive to diallylnortoxiferine. Similarly, the response to repeated applications of diallylnortoxiferine was constant when a 15 min wash out period was used, but the preparation was subsequently more sensitive to tubocurarine. This increase in sensitivity was, however, much less than when tubocurarine preceded diallylnortoxiferine.

A log concentration effect curve for tubocurarine alone was first determined, and the determination was then repeated in the presence of various concentrations of either neostigmine, physostigmine, edrophonium or ambenonium added to the bath 1 min before each dose of tubocurarine. The same procedure was repeated for diallylnortoxiferine. Three or four different concentrations of each antagonist were used, the ranges of which are shown in Table 1.

TABLE 1. RANGE OF MOLAR CONCENTRATIONS OF ANTICURARE AGENTS USED

Anticurare agent	Tubocurarine	Diallylnortoxiferine
Neostigmine	$3{\cdot}33$–$13{\cdot}3 \times 10^{-7}$	$1{\cdot}66$–$6{\cdot}67 \times 10^{-7}$
Physostigmine	$1{\cdot}66$–$6{\cdot}67 \times 10^{-6}$	$0{\cdot}83$–$3{\cdot}33 \times 10^{-6}$
Edrophonium	$1{\cdot}66$–$13{\cdot}3 \times 10^{-6}$	$1{\cdot}66$–$6{\cdot}67 \times 10^{-6}$
Ambenonium	$1{\cdot}66$–$6{\cdot}67 \times 10^{-7}$	$6{\cdot}67$–$26{\cdot}6 \times 10^{-8}$

Using these results, dose ratios for each antagonist were determined and graphs were drawn of log (dose ratio -1) plotted against the negative logarithm of the molar concentration of antagonist (Arunlakshana & Schild, 1959). The slopes of the resulting lines were measured and pA_2 values were derived from the intercept of this plot with the abscissa. For competitive antagonism this plot should result in a straight line of slope $= 1$.

It seemed that ambenonium and edrophonium might antagonize diallylnortoxiferine in a qualitatively different manner to tubocurarine on this preparation. This was investigated by examination of the effect of the duration of pretreatment contact time on the efficiency of the anticurare action of the four antagonists. A 3 min contact time with the neuromuscular blocking agent was allowed and the antagonists were added to the rat phrenic nerve diaphragm preparation 8 min before, 4 min before, and simultaneously with the blocking agent. The doses of antagonist against each neuromuscular blocking drug were selected to give mean responses, at 8 min, between 30 and 70%.

Each mean with its standard error (s.e.) was derived from six experiments. Significance levels were all $P = 0{\cdot}05$ and all statistical calculations were based on the methods of Saunders & Fleming (1957).

Results

Table 2 shows the pA_2 values obtained for each anticurare agent and the slopes of the Arunlakshana & Schild plots against both tubocurarine and diallylnortoxiferine.

TABLE 2. THE POTENCY OF THE ANTICURARE AGENTS EXAMINED

Anticurare agent	pA_2 values (±s.e.)*		A & S** slopes (±s.e.)	
	Tubocurarine	Diallylnor-toxiferine	Tubocurarine	Diallylnor-toxiferine
Neostigmine	5·80 (±0·07)	6·16 (±0·03)	0·80 (±0·09)	0·86 (±0·06)
Physostigmine	4·97 (±0·05)	5·33 (±0·05)	0·74 (±0·08)	0·87 (±0·05)
Edrophonium	4·84 (±0·14)	5·36 (±0·05)	0·47 (±0·11)	0·56 (±0·05)
Ambenonium	5·95 (±0·10)	6·52 (±0·08)	0·49 (±0·07)	0·95 (±0·11)

* All means and standard errors (s.e.) were based on six experiments.
** A & S—Graph of log (dose ratio − 1) plotted against the negative logarithm of the molar concentration of anticurare agent (Arunlakshana & Schild, 1959).

The relative order of potency of the antagonists was the same for tubocurarine and diallylnortoxiferine, ambenonium being most potent, neostigmine second and edrophonium and physostigmine (having no significant difference in their potencies) equal third. All four drugs, however, showed significantly greater antagonistic potency against diallylnortoxiferine than against tubocurarine.

The Arunlakshana & Schild plots gave straight lines in all experiments over the limited range of concentrations used. No experiments were made to determine the range over which this linearity extended, as the necessary pA_2 values and slopes could be obtained from the results plotted. In 25% of experiments some extrapolation was necessary to obtain the pA_2 value but the extrapolation was never more than 10% of the plotted line.

The results of the investigation of the effect of the duration of pretreatment with the antagonists are shown in Figs 1 and 2. It can be seen that, in the presence of both neuromuscular blocking agents, increased time of pretreatment greatly increases the degree of antagonism by neostigmine and physostigmine. This is also true for edrophonium and ambenonium in the presence of diallylnortoxiferine, but in the presence of tubocurarine there is no significant increase in antagonistic potency with time of pretreatment.

The different concentrations of antagonists used against the two neuromuscular blocking agents reflects the increased activity of the antagonists against diallylnortoxiferine as compared with tubocurarine.

Discussion

All the anticurare drugs examined antagonized diallylnortoxiferine more than tubocurarine. This correlates with the clinical findings of Venn (1965) and Foldes & others (1962), but not with those of Hunter

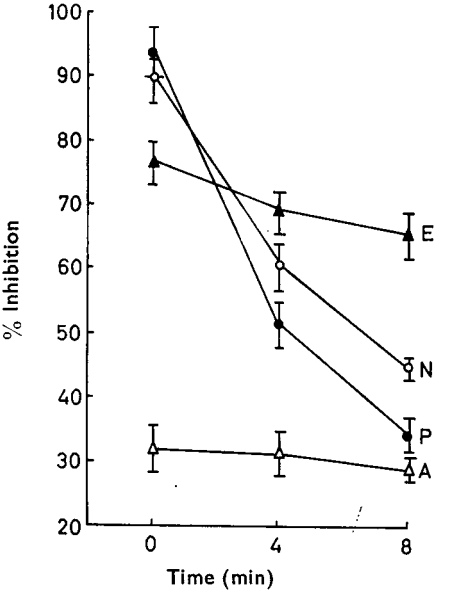

FIG. 1. The effect of the time of pretreatment on the efficiency of tubocurarine antagonism by four anticurare drugs. E = $1{\cdot}0 \times 10^{-6}$M edrophonium. N = $8{\cdot}45 \times 10^{-8}$M neostigmine. P = $6{\cdot}67 \times 10^{-7}$M physostigmine. A = $6{\cdot}67 \times 10^{-8}$M ambenonium. Ordinate represents % inhibition relative to tubocurarine $3{\cdot}33 \times 10^{-6}$M = 100%.

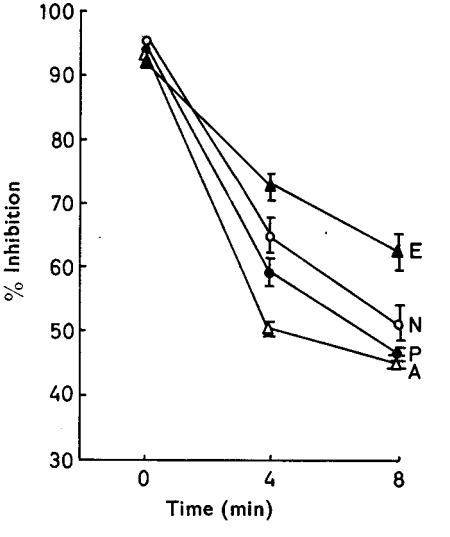

FIG. 2. The effect of the time of pretreatment on the efficiency of diallylnortoxiferine antagonism by four anticurare drugs with s.e. at time 0. E = $6{\cdot}67 \times 10^{-7}$M edrophonium ($\pm 2{\cdot}77$); N = $6{\cdot}67 \times 10^{-8}$M neostigmine ($\pm 2{\cdot}32$); P = 5×10^{-7}M physostigmine ($\pm 3{\cdot}375$); A = $3{\cdot}33 \times 10^{-8}$M ambenonium ($\pm 2{\cdot}46$). Ordinate represents % inhibition relative to tubocurarine $3{\cdot}33 \times 10^{-6}$M = 100%.

(1964) (who found reversal uncertain with diallylnortoxiferine), or of Lund & Stovner (1962) (who found no difference in the reversibility of the two relaxants). It must be remembered, however, that post-treatment with the antagonist is used clinically, not pretreatment as was used in the present work.

The relative anticurare potencies of the four antagonists do not correlate with any recorded value for relative anticholinesterase potencies (Table 3). But if figures could be obtained for the activity of these drugs against the cholinesterase present in rat diaphragm, more meaningful comparisons might be made.

TABLE 3. THE RELATIVE ANTICHOLINESTERASE POTENCIES OF THE DRUGS EXAMINED

Agonist	Potency ratio				References
	Neostigmine	Physo-stigmine	Edroph-onium	Ambenonium	
Tubocurarine	1	0·145	0·089	1·3	
Diallylnortoxiferine	1	0·155	0·165	2·34	
Cholinesterase of cat CNS	1	0·5			Bhattacharya & Feldberg (1958)
Red blood corpuscles	1	0·58	<0·01	5·8	Lands, Hoppe & others (1958)
Bovine red blood corpuscles	1	0·88	0·02		Smith, Cohen, & others (1952)
Red blood corpuscles	1		0·009		Hobbiger (1952)
Cat anterior tibialis homogenate	1	0·5	0·01	35	Blaber (1963)
Dog caudate nucleus	1	0·5			Blaschko, Bulbring & Chou (1949)

When tubocurarine was the agonist, the slopes obtained from the Arunlakshana & Schild plots suggested a division of the four anticurare drugs into two groups. The slopes of the plots for neostigmine and physostigmine were not significantly different and the slope for neostigmine was not significantly different from 1. Similarly the slopes for edrophonium and ambenonium were not significantly different from each other, but were significantly different from those of physostigmine and neostigmine. Thus neostigmine and physostigmine almost fulfil the requirements for competitive antagonism, but edrophonium and ambenonium do not. This difference is not so marked for antagonism to diallylnortoxiferine, only edrophonium producing an Arunlakshana & Schild slope significantly different from 1. However, edrophonium and ambenonium do not have the same quantitative relations between concentration of anticurare drug and potency as physostigmine and neostigmine. There may therefore be a qualitative difference in their anticurare mechanism at least against tubocurarine.

The differences in the time course of tubocurarine antagonism by neostigmine and edrophonium have been investigated by Smith, Mead & Unna (1957). They postulated that, in the intact animal, differences in the time effect curve obtained for the antagonism to curare produced by these compounds are causally related to the differences in the kinetics of the inhibitor-cholinesterase combinations and dissociations. Thus, if it is accepted that anticholinesterase activity plays at least a part in the

anticurare mechanism of the four anticurare drugs examined, this hypothesis could equally well apply to the time course differences between physostigmine and neostigmine on the one hand and edrophonium and ambenonium on the other.

However, there is no obvious explanation of why the time courses for all four drugs are similar against diallylnortoxiferine, but not against tubocurarine. This may indicate a qualitative, as well as quantitative difference between the relaxant drugs.

The nature of this possible difference and the differences between the two groups of anticurare drugs has not been revealed.

An attempt was made to demonstrate anticurare activity with the four antagonists in a preparation in which all the cholinesterase had previously been inactivated by contact with di-isopropylfluorophosphonate (DFP) 20 μg/ml for 1 hr. After application of this drug it was not possible to demonstrate antagonism of tubocurarine by any of the antagonists even when concentrations were increased. This would at first sight suggest that all the antagonists were simply anticholinesterase agents. However, Webb (1948) showed that DFP also inhibited a number of other enzyme systems; Burgen, Keele & Slome (1949) demonstrated the direct action of DFP on the motor end-plate and Riker, Roberts & others (1957) suggested that DFP may have some presynaptic activity, and so this argument is not wholly conclusive.

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