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Anticurare activity of tacrine (THA) *in vitro*

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Tacrine (THA) has been found to be a relatively weak anticurare agent having a significant anticurare activity on the rat isolated phrenic nerve diaphragm preparation in concentrations above 10^{-7} M. It is about 20 times less potent than neostigmine. Between 10^{-5} M and 10^{-4} M, anticurare activity of THA ceases to be reproducible, and in concentrations above 10^{-4} M the compound exerts a depressant action directly on the muscle. The qualitative and quantitative differences between the anticurare activities of THA and neostigmine are discussed.

ALTHOUGH the anticurare activity of tacrine (THA) has been demonstrated *in vivo* and *in vitro* (Gershon & Shaw, 1958), no quantitative estimation of its potency *in vitro* has been made.

A quantitative comparison has now been made between the anticurare activities of THA and neostigmine on the rat isolated phrenic nerve diaphragm preparation.

Bentley & Shaw (1949, 1953) showed THA to be a respiratory stimulant and they also reported "profound parasympathetic stimulation". They later showed THA to be an anticholinesterase of the same order of potency as neostigmine and eserine, and this has since been confirmed (Heilbronn, 1961; Ho & Freeman, 1965).

Experimental

METHODS

The preparation used was essentially that described by Bülbring (1946). Female rats, 200–250 g, were used. The tissue baths of 15 ml capacity were identical to those used by Starmer & Thomas (1961). The preparation was maintained in Krebs solution at 38° gassed with a 95% oxygen, 5% carbon dioxide mixture. The preparation was so arranged that it could be stimulated directly or by way of the phrenic nerve. Rectangular electrical pulses of 2·0 to 2·5 msec duration and of a strength just greater than that necessary to evoke maximal twitches were applied at a frequency of 6/min. The contractions were recorded by a Brodie Universal writing lever on a smoked drum.

The drugs used were (+)-tubocurarine chloride, neostigmine methyl sulphate, and 5-amino-1,2,3,4-tetrahydroacridine hydrochloride (tacrine, THA).

In all instances concentrations are expressed as final bath molar concentrations.

Results

Two series of experiments were made. In the first the anticurare agent was added to the bath fluid before or with the tubocurarine, and in the

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second the anticurare agent was added during partial block produced by tubocurarine.

The first series of experiments was designed according to the method of Bülbring & Chou (1947). A log dose response curve for tubocurarine was determined and this was then repeated in the presence of either THA or neostigmine. THA or neostigmine was added to the bath 5, 2, or 1 min before each dose of tubocurarine, or the tubocurarine and the antagonist were added together. The largest dose of each antagonist investigated was about 40 times bigger than the smallest. The antagonism was expressed as the molar concentration of anticurare agent that reduced the effect of a dose of tubocurarine to that of a dose half the size. These values are termed A_2 values (Schild, 1947) and are given in Table 1. The standard deviations of the A_2 values were usually small for experiments done on the same preparation but in different preparations the A_2 values for THA varied sometimes by as much as 100%. With neostigmine there was no more variation between preparations than there was in the same preparation.

TABLE 1. THE RELATIVE ACTIVITIES (± S.D.) OF NEOSTIGMINE AND THA AS ANTICURARE AGENTS

Pretreatment time with anticurare agent	A_2 value		Potency ratio, where activity of THA for 5 min pretreatment = 1·0	
	THA	Neostigmine	THA	Neostigmine
5 min	$1{\cdot}379\ (\pm 0{\cdot}493) \times 10^{-6}$ (4)*	$2{\cdot}610\ (\pm 0{\cdot}123) \times 10^{-8}$ (4)	1·0	54·0
2 min	$1{\cdot}568\ (\pm 0{\cdot}092) \times 10^{-6}$ (5)	$6{\cdot}123\ (\pm 1{\cdot}48) \times 10^{-8}$ (5)	0·9	22·5
1 min	$6{\cdot}264\ (\pm 1{\cdot}32) \times 10^{-6}$ (4)	$3{\cdot}596\ (\pm 1{\cdot}43) \times 10^{-7}$ (4)	0·2	3·8
Simultaneous addition	Could not be determined	$2{\cdot}437\ (\pm 0{\cdot}946) \times 10^{-7}$ (5)	—	5·7

The A_2 value is the molar concentration of anticurare agent that would reduce the effect of a double dose of tubocurarine to that of a single dose.

* Figures in parenthesis indicate number of experiments.

In the second series of experiments, the bath concentration of tubocurarine produced each time was $1{\cdot}4 \times 10^{-7}$ M. Either 2 or 4 min after the addition of tubocurarine, the anticurare agent was added and the extent of the antagonism of the block was measured either 2 or 4 min later respectively. Two concentrations of each anticurare agent were used. For THA these were $4{\cdot}2$ and $2{\cdot}1 \times 10^{-6}$ M and for neostigmine, $9{\cdot}2$ and $4{\cdot}6 \times 10^{-7}$ M. The doses of each anticurare agent were added to the bath according to a Latin square design. When the 2 min contact time was used, the log dose response slopes for THA and neostigmine did not differ significantly from parallelism. The ratio of anticurare potencies was found to be neostigmine, 5·77 : THA, 1·00 ($P = 0{\cdot}05$, range of standard deviation 5·65–5·88).

When the 4 min anticurare contact time was used the partially blocked contractions were initially increased in strength by THA but the effect began to wane after about 2 min and the responses became erratic.

At concentrations near 10^{-4} M, THA depressed both directly and indirectly elicited contractions. A concentration of 10^{-4} M produced a

30% depression in 10 min and a concentration of $4{\cdot}3 \times 10^{-4}$ M abolished the twitches within 10 min. Neither potassium ion nor neostigmine reversed the effect.

The depression was rarely quantitatively reproducible after washing the preparation.

The mechanism of this action cannot be restricted to the neuromuscular junction.

Discussion

An A_2 value is indicative only of a small degree of antagonism, and Table 1 shows that an A_2 value could not be determined for simultaneous addition of THA; this could be because at concentrations above 10^{-5} M the anticurare activity of THA is antagonised by a direct inhibitory effect on the muscle. This effect has also been reported by Gershon & Shaw (1958).

Table 1 shows that at 5 min pretreatment neostigmine was over 50 times more potent than THA, and this ratio falls progressively so that at 1 min pretreatment neostigmine is only 17 times more potent. It has been established that the activity of anticurare agents decreases as the interval between their administration and that of tubocurarine decreases (Bülbring & Chou, 1947). From the ratio of potencies it will be seen that the activity of neostigmine falls by a greater fraction than the activity of THA.

On post-treatment, using a 2 min anticurare contact time, neostigmine was found to be only six times more potent than THA. However using the 4 min anticurare contact time, THA was less potent, for although its anticurare action appeared quickly, the effect was only transient. THA therefore appears to have a shorter duration of action than neostigmine.

It has been shown in man that THA is a relatively ineffective decurarising agent compared with neostigmine, particularly when there was profound curarisation (Hunter, 1965). The ratio of intravenous doses in man for decurarisation is neostigmine, 1 : THA, 15. It is of interest that these relative potencies are similar *in vivo* and *in vitro*.

THA is an inhibitor of acetylcholinesterase equipotent with neostigmine (Heilbronn, 1961), though it is a more potent inhibitor of pseudocholinesterase (Ho & Freeman, 1965). The ability of an anticholinesterase to reverse curarisation is believed to be due primarily to inhibition of acetylcholinesterase, though this has been the subject of some controversy (for references see Barlow, 1964).

With THA, its anticurare activity is less potent than its anticholinesterase activity would indicate. Porter (1965) has attributed the potentiation of acetylcholine on the toad rectus solely to inhibition of cholinesterase, and in this preparation THA is equipotent with neostigmine. There is therefore a strong indication that on the rat isolated phrenic nerve diaphragm preparation there is some essential difference between the anticurare activity of neostigmine and THA, for THA differs from neostigmine in its potency, time course of action, and the presence of an interfering action.

NOTE ON THE APPLICATION OF pA_x VALUES TO CURARE-ANTICURARE ANTAGONISM

In 1947 Schild introduced and discussed the pA_x value as a measurement of antagonist activity. The applicability of such a criterion to the present situation may be challenged on two counts. Firstly the degree of antagonism is not measured at equilibrium but at a fixed time known to be short of equilibrium. Secondly, curare-anticurare antagonism is probably not a simple antagonism at one receptor, but involves two distinct systems namely the cholinesterase site and the nicotinic receptor.

In an attempt to differentiate between the nature of neostigmine and THA curare antagonism, pA_2 values were calculated both after the method of Schild (1947), and from the graph of log dose ratio -1 plotted against the negative logarithm of the molar concentration of antagonist (Arunlakshana & Schild, 1959). From the slope of this line, the $pA_2 - pA_{10}$ value should equal approximately 0·95 for competitive antagonism. The results are given in Table 2.

TABLE 2. A COMPARISON OF THE pA_2 VALUES OF THA AND NEOSTIGMINE ANTAGONISM OF TUBOCURARINE CALCULATED AFTER SCHILD (1947) (i), AND ARUNLAKSHANA & SCHILD (1959) (ii)

Drug	Pretreatment min	pA_2 (i) (± s.d.)	pA_2 (ii)	$pA_2 - pA_{10}$
Neostigmine	simultaneous	6·64 (±0·18)	6·58	1·47
	1	6·47 (±0·19)	6·74	1·03
	2	7·22 (±0·10)	7·15	1·33
	5	7·58 (±0·22)	7·58	0·81
THA	simultaneous*		4·05	4·10
	1	5·22 (±0·08)	5·23†	2·60
	2	5·80 (±0·03)	5·56†	2·12
	5	5·88 (±0·17)	5·88	1·39

* pA_2 value (i) could not be determined practically. pA_2 (ii) and $pA_2 - pA_{10}$ calculated by extrapolation
† Denotes that the points do not lie on a straight line. These figures are therefore approximate.

For curare-anticurare antagonism to behave as true competitive antagonism, the concentration of anticholinesterase must be directly proportional to the additional acetylcholine available to compete with tubocurarine for the nicotinic receptors. However, the degree of antagonism is limited by the availability of acetylcholine.

It will be seen that neostigmine almost fulfils the requirements for competitive antagonism at all the time intervals investigated over a 40-fold increase in concentration. On the other hand, with THA this only occurs for 5 min pretreatment at a concentration of 10^{-6} M. Were THA merely a weaker anticholinesterase agent then it should still fulfil exactly the same requirements as does neostigmine.

The value of a pA_2 determination is that it is independent of agonist concentration, and is reproducible. It is of interest that if the results of Bülbring & Chou (1947) for neostigmine and tubocurarine are calculated in terms of pA_2 and $pA_2 - pA_{10}$, values close to the present ones emerge: 6·80 compared with the present study 6·74, and 0·95 compared with the present study 1·03 respectively. The neostigmine was added to the bath

1 min before tubocurarine and the reversal (%) of curarisation measured after 3 min.

Thus even taking into account theoretical criticisms of the application of pA_2 to curare-anticurare antagonism, it is evident that there is a large difference between THA and neostigmine as anticurare agents.

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