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STRUCTURE-ACTIVITY RELATIONSHIPS FOR THE ANTICHOLINOCEPTOR ACTION OF TRICYCLIC ANTIDEPRESSANTS

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1 The anticholinoceptor action of 15 tricyclic antidepressants and derivatives has been studied on the guinea-pig ileum. At the muscarinic receptors the compounds were found to exert antagonism which was reversible and apparently competitive up to dose-ratios of around 100 but non-competitive above this level.

2 Log affinity constants were derived from log dose-response curves at dose-ratios <100, where parallel curves were obtained. Amitriptyline, the most potent compound, had 214× the potency of the weakest, hydroxyimipramine, but was itself 20× weaker than atropine.

3 Structure-activity studies showed that dibenzocycloheptane derivatives were more potent than dibenzazepines and that S or O substitution for C-11 or other major changes in the central ring of the tricyclic nucleus greatly reduced activity. Side-chain N-methylation increased potency markedly. This and other findings indicate that both tricyclic nucleus and side-chain receptor attachments are largely non-polar in type.

Introduction

It is well known that tricyclic antidepressants have anticholinoceptor actions on both the central nervous system (Loew & Taeschler, 1965; Ho, Freeman, Freeman & Lloyd, 1966; Gupta, Gupta & Bhargava, 1967) and peripheral organs (Theobald, Buch & Kunz, 1965; Brimblecombe & Green, 1967; Atkinson & Ladinsky, 1972). Such actions are responsible for marked side-effects in clinical practice but may also play a part in determining the therapeutic effects produced.

The aims of this study were to evaluate the type of antagonism exerted by tricyclic antidepressants and to examine structure-activity relationships for their anticholinoceptor action on the muscarinic receptors of the isolated ileum of the guinea-pig.

Methods

General aspects

Terminal guinea-pig ileum was suspended in Tyrode solution at 37°C and aerated with 5% CO_2 in oxygen.

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With carbachol as agonist, isotonic contractions were elicited under 1.5 g tension using a 2 min cycle, each contraction being allowed to reach a maximum.

Agonist log dose-response curves were obtained in the absence and presence of each of 15 tricyclic antidepressants and derivatives at 2 to 4 different concentrations. Affinity constants were determined by the method of Arunlakshana & Schild (1959) using dose-ratios of <100 because of the apparent non-competitive nature of the antagonism exerted by these compounds. In each case the antagonist was allowed to reach equilibrium with the tissue (usually >30 minutes). Best fit regression lines for plots of log (dose-ratio $-$ 1) v. negative log antagonist concentration (Schild, 1957) were obtained by the method of least squares and the affinity constants with 95% confidence limits by interpolation (Snedecor & Cochran, 1967).

Reversibility of antagonism was tested in a series of experiments with acetylcholine and carbachol as agonists and nortriptyline as antagonist. Time was allowed for the antagonist to reach equilibrium with the tissue as above, after which repeated doses of agonist were applied and washed out over the next 3 hours.

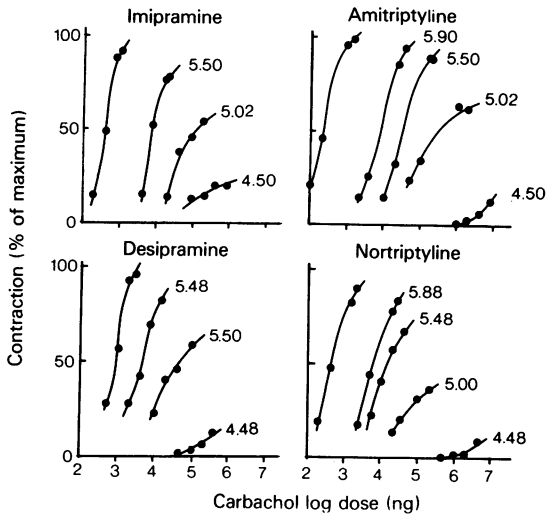


Figure 1 Carbachol log dose-response curves on guinea-pig ileum in the absence and presence of tricyclic antidepressants and analogues. Values against graphs indicate negative log M concentrations of antagonists.

Drugs

The compounds used were: acetylcholine HCl (Lematte & Boinot); carbachol (Sigma); imipramine HCl, chlorimipramine HCl, desipramine, HCl, 2-hydroxyimipramine HCl, desmethylchlorimipramine HCl and didesmethylimipramine HCl (Geigy); amitriptyline HCl and protriptyline HCl (Merck Sharpe & Dohme); dothiepin (Boots); doxepin HCl (Pfizer); iprindole HCl (Wyeth); nortriptyline HCl (Eli Lilly); trimipramine maleate and N-desmethyltrimipramine maleate (May & Baker); and dibenzepin HCl (Sandoz).

Results

Anticholinoceptor action

All the tricyclic antidepressants and analogues studied were found to possess anticholinoceptor activity, as detected by displacement of carbachol log dose-response curves to the right. At concentrations which caused only small or modest degrees of antagonism, the log dose-response curves were parallel, but at high concentrations the curves were flattened and disproportionately displaced downwards and to the right. Four examples are illustrated in Figure 1. In no case was parallel displacement found beyond a dose-ratio of 100. Such an appearance, which is characteristic of non-competitive antagonism, was found in all but one instance of a compound which was one of the weakest antagonists (dibenzepin). With all drugs and at all concentrations antagonism attained a steady level at about 30 to 45 minutes.

Antagonism by nortriptyline was found to be slowly reversible, as illustrated in Figure 2. However, in this and other replicate experiments recovery was incomplete (80–90%) even at 3 h, particularly in response to carbachol as agonist.

Assessment of the antagonist affinity for the recep-

Table 1 Anticholinoceptor action of tricyclic antidepressants

Drug	n	*Slope**	*Log affinity constant*	*95% Confidence limits*	*Potency relative to imipramine (=100)*
Amitriptyline	9	−1.04	7.66	7.37–7.99	424
Protriptyline	8	−1.20	7.38	7.14–7.62	224
Trimipramine	8	−1.10	7.22	7.18–7.26	152
Dothiepin	6	−0.96	7.19	6.89–7.52	142
Nortriptyline	13	−1.08	7.11	6.94–7.30	118
Chlorimipramine	17	−1.12	7.05	6.86–7.24	102
Imipramine	18	−1.00	7.04	6.64–7.47	100
Doxepin	19	−1.12	6.66	6.26–7.09	42
Desmethylchlorimipramine	10	−1.18	6.66	6.46–6.89	42
Desipramine	13	−1.11	6.56	6.26–6.87	33
N-desmethyltrimipramine	6	−0.96	6.47	6.54–7.01	27
Desdimethylimipramine	5	−1.29	5.95	5.72–6.16	8
Dibenzepin	9	−1.01	5.80	5.61–6.00	6
Iprindole	5	−0.84	5.63	5.00–5.85	4
Hydroxyimipramine	6	−1.16	5.33	5.19–5.47	2

* log (DR − 1) v. neg. log M concentration.

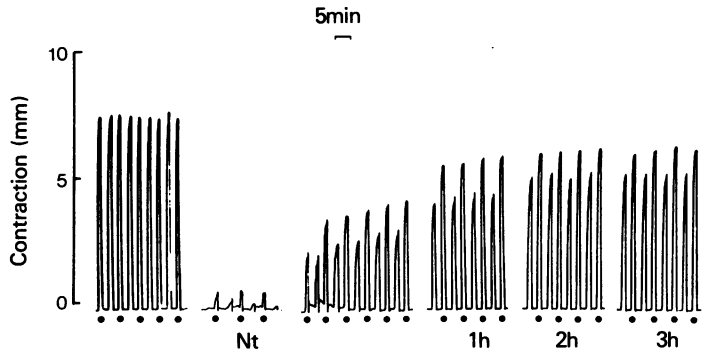


Figure 2 Responses of guinea-pig ileum to acetylcholine (identified by dot) and carbachol before, during and after exposure to nortriptyline 3.3×10^{-6} M (Nt) for 30 minutes.

tor was based on measurements of antagonism only where parallel displacement occurred (i.e. at DR <100). A graphic presentation of the relationship between log (DR − 1) and negative log M antagonist concentration for all 15 compounds is shown in Figure 3. Values for the line slope, mean calculated affinity constant, its confidence limits and the potency relative to imipramine are given in Table 1. The most potent compound, amitriptyline, had 214× the potency of the weakest, hydroxyimipramine, but was itself 20× weaker than atropine.

Structure-activity relationships

The influence of structural changes in the tricyclic nucleus on anticholinoceptor potency is shown in

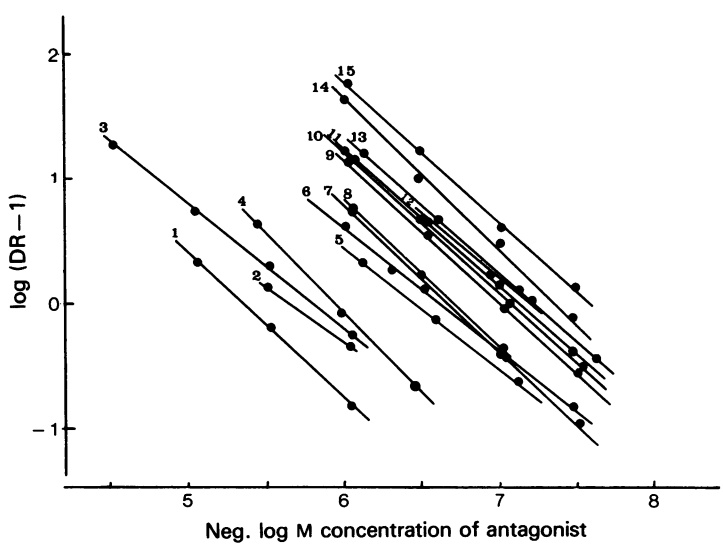
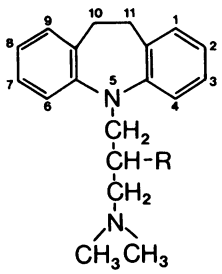


Figure 3 Antimuscarinic action of tricyclic antidepressants and analogues on the isolated ileum of the guinea-pig. Plot of log (DR − 1) v. negative log M concentration of antagonist. Each point is the mean of 2 to 5 (usually 3 or 4) determinations. The identity of the lines are: 1: 2-hydroxyimipramine. 2: Iprindole. 3: Dibenzepin. 4: Desdimethylimipramine. 5: N-desmethyltrimipramine. 6: Desipramine. 7: Desmethylchlorimipramine. 8: Doxepin. 9: Imipramine. 10: Chlorimipramine. 11: Nortriptyline. 12: Dothiepin. 13: Trimipramine. 14: Protriptyline. 15: Amitriptyline.

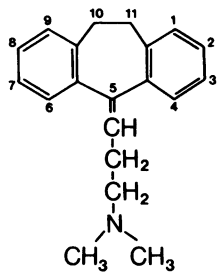
Table 2. The following structural formulae are provided for reference.

Dibenzazepine



Imipramine, R=H
Trimipramine, R=CH_3

Dibenzocycloheptane



Amitriptyline

The dibenzocycloheptane derivatives were found to be generally more potent than the dibenzazepine derivatives. Thus amitriptyline and nortriptyline differed from imipramine and desipramine by factors of 4.17 and 3.50 fold respectively. In the dibenzazepine series, the presence of chlorine on carbon 3 did not significantly change potency, but hydroxylation of carbon 2 reduced activity greatly. In the dibenzocycloheptane series, substitution of C at the 11 position with S or O decreased activity, O causing a greater decrease than S. Unsaturation at 10-11 and C-5 saturation as in protriptyline increased potency. Dibenzepin and iprindole, which have atypical ring systems, were 70- and 100-fold weaker than amitriptyline.

The influence of methylation of the propylene side chain is shown in Table 3. Methylation of the terminal nitrogen increased anticholinoceptor potency, the secondary amine desipramine being more active than its primary amine desdimethylimipramine, and the tertiary amines amitriptyline and imipramine being more potent than their corresponding secondary amines ($P < 0.01$).

Beta-methylation of the side chain was found to have negligible influence. In the pair of secondary amines, N-desmethyltrimipramine and desipramine, there was a slight decrease in potency, whereas in the pair of tertiary amines the potency was increased 1.5-fold.

Table 2 Effects of substitution on or within the tricyclic nucleus on anticholinoceptor potency

Substituent	*Parent drug (P)*	*Derivative (D)*	*Potency ratio* (D/P)*
3-Cl	Imipramine	Chlorimipramine	1.02
	Desipramine	Desmethylchlorimipramine	1.26
2-OH	Imipramine	Hydroxyimipramine	0.02
C at 5 and bond unsaturation	Imipramine	Amitriptyline	4.17
	Desipramine	Nortriptyline	3.50
S at 11	Amitriptyline	Dothiepin	0.34
O at 11	Amitriptyline	Doxepin	0.10
10-11 unsaturation & C-5 saturation	Nortriptyline	Protriptyline	1.86

* Ratio of affinity constants, derived from Table 1.

Table 3 Effects of side chain methylation on anticholinoceptor potency

Substituent	*Parent drug (P)*	*Derivative (D)*	*Potency ratio* (D/P)*
N-methylation	Desdimethylimipramine	Desipramine	4.07
	Desipramine	Imipramine	3.02
	Desdimethylimipramine	Imipramine	12.30
	Desmethylchlorimipramine	Chlorimipramine	2.45
	N-desmethyltrimipramine	Trimipramine	5.62
	Nortriptyline	Amitriptyline	3.55
β-Methylation	Desipramine	N-desmethyltrimipramine	0.81
	Imipramine	Trimipramine	1.52

* Ratio of affinity constants, derived from Table 1.

Discussion

The anticholinoceptor action of tricyclic antidepressants has been recognized since the early studies of Sigg (1959) but there have been few quantitative studies on this property of the drugs. Studies of Atkinson & Ladinsky (1972) on the rat fundus and those of Theobald *et al.* (1965) and Brimblecombe & Green (1967) on the isolated ileum of the guinea-pig, provided quantitative data on only a few drugs that are now in clinical use. The present study on 15 drugs and derivatives indicates that antagonism on muscarinic receptors is at least partly non-competitive in type, as shown by the non-parallelism of agonist log dose-response curves at high dose-ratios. It is probable, therefore, that as antagonists the tricyclic compounds do not attach themselves to the receptor site in the same manner as do agonists. The experiments show further that the attachment is reversible because, in the presence of the antagonist, equilibrium conditions were reached in about 30 min and because washout of the antagonist resulted in 80–90% restoration of the agonist response within 2–3 hours.

It is apparent from the structure-activity relationships that antagonism by tricyclic compounds has marked structural requirements for maximal activity, changes in both tricyclic skeleton and side chain exerting profound effects. The salient features are as follows. Major alteration of the nucleus as in iprindole and dibenzepin or substitution of sulphur or oxygen for carbon in the central ring reduced activity, as did hydroxylation at carbon 2 which increases polarity of this part of the molecule. Optimal activity therefore requires the presence of a lipophilic nucleus of the conventional type. Comparison of the dibenzocycloheptanes and dibenzazepines revealed a surprising dependence on carbon 5 in the former, perhaps because the resultant double bond attachment of the side chain at this position confers increased conformational rigidity on the side chain itself. Methylation of the side chain amine group was found to enhance activity about 3 fold, but methylation on the β-carbon had little influence. Amine substitution does not alter ionisation of the nitrogen as reflected in pK_a values of these compounds: viz. desipramine 9.5, imipramine 9.5 (Smith & Rawlins, 1973). It must therefore be assumed that, despite the high percentage ionisation of this group at the pH of the Tyrode solution, attachment of the terminal part of the side chain is largely non-polar in type.

The experiments described here give no indication of the specificity of the antagonism produced by these compounds. Other experiments show, however, that nortriptyline has H_1-antihistaminic properties and that all the compounds possess some degree of local anaesthetic activity (unpublished observations). It seems likely therefore that at least some of the anticholinoceptor activity investigated is non-specific.

As muscarinic antagonists, tricyclic compounds are weak by comparison with atropine (log K = 9.00, Arunlakshana & Schild, 1959). Their high dosage employed in clinical practice, however, makes comparable effects likely. Unfortunately, the published literature does not permit valid comparisons of anticholinoceptor actions between the various tricyclic antidepressants under clinical conditions.

We thank the pharmaceutical companies mentioned for generous gifts of tricyclic compounds. K.S. is a W.H.O. Fellow. Some of the work was done in part fulfilment by K.S. of a Ph.D. thesis of the University of London.

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