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# A quantitative study of the anticholinergic action of several tricyclic antidepressants on the rat isolated fundal strip

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## Summary

1. Investigations were carried out on the antagonism of the action of carbamylcholine chloride on the isolated fundus of the rat stomach by several tricyclic antidepressants.

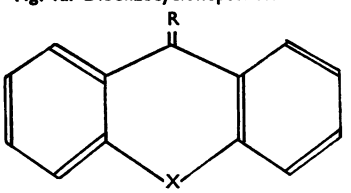
2. The anticholinergic potency of the compounds was in the order: GP 45437>amitriptyline>protriptyline>desmethylimipramine>opipramol. All of the antagonists were less effective than atropine.

3. Statistical analysis was carried out to determine whether the theory of competitive antagonism would fit the data obtained.

## Introduction

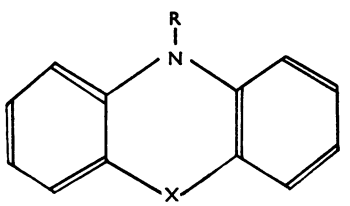
Since the work of Sigg (1959) on the anticholinergic activity of imipramine, there have been several reports on the peripheral and central anticholinergic effects of tricyclic compounds (Domenjoz & Theobald, 1959; Biel, Nuhfer, Hoya, Leiser & Abood, 1962; Sulser, Bickel & Brodie, 1964; Theobald, Büch, Kunz, Morpurgo, Stenger & Wilhelmi, 1964; Theobald, Büch & Kunz, 1965, and Aichinger, Behner, Hoffmeister & Schütz, 1969). In all this work the anticholinergic properties have been quantitatively expressed relative to one another. However, we could find no

Fig. 1a. Dibenzocycloheptenes:




| Compound | R= | —X— |
|---|---|---|
| GP 45 437 | $=CHCH_2CH_2N(CH_3)_2$ | —C=CH— (CH₃) |
| Amitriptyline | $=CHCH_2CH_2N(CH_3)_2$ | $—CH_2—CH_2—$ |
| Protriptyline | $=CHCH_2CH_2NHCH_3$ | —CH=CH— |

Fig. 1b. Dibenzazepines




| Compound | R— | —X— |
|---|---|---|
| DMI | $—CH_2CH_2CH_2NHCH_3$ | $-CH_2-CH_2-$ |
| Opipramol | $—CH_2CH_2CH_2—N\square N—CH_2CH_2OH$ | -CH=CH- |

FIG. 1. Structure of tricylic compounds

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information on the inhibitory action of these compounds based on the theories of competitive and non-competitive antagonism developed from the work of Schild (1947), Gaddum, Hameed, Hathway & Stephens (1955) and others.

In the work reported here, the atropine-like effects of the derivatives of dibenzazepines (desmethylimipramine (DMI) and opipramol) and dibenzocycloheptene (amitriptyline, protriptyline and GP 45437 (a methylated dibenzocycloheptene)) (see Fig. 1 and Klerman & Cole, 1965) have been quantitatively investigated.

## Methods

### *Preparation of fundal strips*

Female, Sprague-Dawley rats of 150–190 g body weight were killed by decapitation and rat fundal strips were prepared as described by Vane (1957). Two strips were cut from each fundus and each strip was mounted in a 25 ml bath containing modified Krebs solution (NaCl, 119·8 mM; KCl, 4·6 mM; $CaCl_2$, 2·5 mM; $MgSO_4$, 0·57 mM; $NaHCO_3$, 2·5 mM; $KH_2PO_4$, 1·2 mM and glucose, 10·9 mM) maintained at 37° C. When gassed with 95% $O_2$:5% $CO_2$, this solution had a pH of 7·35. A constant 1 g tension was used except during the first 30 min after mounting when an extra 1 g load was applied. After removal of the extra load, the tissue was allowed to equilibrate for 1 h before the experiment proper was started. Under these conditions the strips were under zero or minimal tone. Contractions were recorded on a kymograph by frontal, ink-writing, isotonic levers with five-fold amplification.

### *Experimental procedure*

After obtaining at least two identical dose-response curves with carbamylcholine chloride (carbachol) as agonist, the antagonist was added and allowed to equilibrate in the bath for 10 min before the final dose-response curve was determined. The final concentration of carbachol in the bath in the absence of antagonist was $0{\cdot}93\text{–}1{\cdot}30 \times 10^{-6}$ mol/litre.

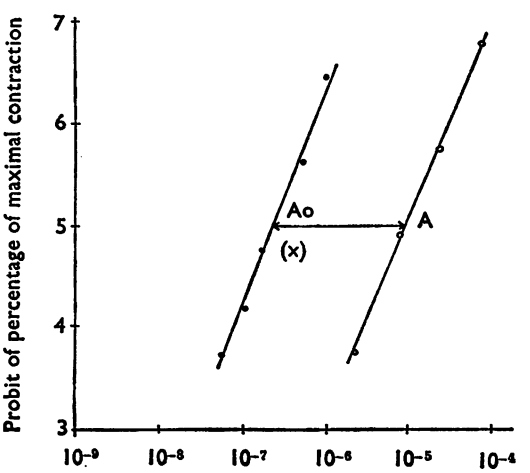


FIG. 2. Log concentration of agonist

### *Statistical analysis*

After an initial test of its validity, a conventional probit transformation (Finney, 1962) was applied to the percentage maximal contraction values. From a straight line plot (drawn by eye) of the probit values versus the logarithm of the agonist concentration, the value of the latter for a probit value of 5 was taken as the carbachol concentration required to produce 50% of the maximal contraction, in the presence (A) or absence (Ao) of a concentration (B) of the antagonist (see Fig. 2; notation as Gaddum *et al.*, 1955). From the values so obtained the dose-ratio was calculated as: dose-ratio (x)=(Ao/A). Dose-ratios in the range of 3 to 170 were obtained. pAx, which has been defined as 'negative logarithm (to base 10) of the molar concentration of an antagonistic drug which will reduce the effect of a multiple dose of an active drug to that of a single dose' (Schild, 1947), was calculated as: pAx=$-\log(B)$, where (B) is the antagonist concentration in mol/litre giving a shift in dose-ratio of (x).

If the conditions of competitive antagonism are fulfilled, a plot of $\log(x-1)$ against pAx gives a straight line with a slope equal to $-1$. In order to test whether this theory was statistically applicable, the data for each drug were subjected to a weighted regression analysis in which the dependent variable (Y) was $\log(x-1)$ and the independent variable (X) was pAx. The weight (W) applied to each Y value was: $W=1/s^2$ where $s^2$ is the estimate of the variance of the observations at the corresponding X. For the first order regression: $Y=\alpha+\beta X$, 'a' and 'b' were taken as the estimates of $\alpha$ and $\beta$.

An analysis of variance of the weighted regression was carried out and this allowed the following tests to be made:

*(a) Test for regression:* $F_R=\dfrac{\textit{mean square (MS) regression}}{\textit{MS deviation}}$

If this is significant when compared to tabulated F values then so is the regression and the slope is considered to be different from 0. The following convention for probability was used (see Table 1) NS: $P>0{\cdot}05$, S: $P\leqslant 0{\cdot}05$, VHS: $P\leqslant 0{\cdot}001$.

*(b) Test for linearity:* $F_D=\dfrac{\textit{MS deviation}}{\textit{MS residual}}$

If this is not significant when compared to tabulated F values, then the linear model is accepted.

*(c) Test of slope*

To test the hypothesis that: $b=-1$, $t$ is calculated from a comparison of the actual 'b' value obtained with the theoretical value of $-1$. The $t$ value so calculated is compared to tabulated values of $t$ and if found to be not significant then the hypothesis that 'b' was not different from $-1$ is accepted.

$pA_2$ was calculated as Xo, i.e., the value of X when $Y=0$. The confidence interval associated with Xo was calculated by the usual method. The results obtained from the various statistical tests are given in Table 1.

### *Drugs used*

The drugs used were desmethylimipramine hydrochloride, opipramol hydrochloride, GP 45437 fumarate (Geigy), amitriptyline hydrochloride, atropine sul-

phate (Hoffmann-La Roche), protriptyline hydrochloride and carbamylcholine chloride (Merck).

## Results

In Fig. 2 is shown a typical probit-transformed cumulative dose-response curve for carbachol before and after the addition of antagonist, in this particular case $1{\cdot}73 \times 10^{-7}$ mol/litre GP 45437. A graphical interpretation of the symbols given in the **Methods** section is also given. Ao and A were taken from the graph and x, log(x−1) and pAx were calculated. The average Ao value for carbachol was $2{\cdot}08 \pm 0{\cdot}10 \times 10^{-7}$ mol/litre (mean of 106 experiments).

In Table 1 are given the values for the slope and $pA_2$ (Xo) plus the 95% confidence interval for each antagonist. It was found that concentrations of atropine and opipramol greater than those necessary to produce a 49× and a 70× change in dose-ratio respectively, gave data which did not fit the hypothesis applied. Otherwise, for all drugs, over the dose-ratio range given, the model was found to be valid and the hypothesis correct, and consequently the theory of competitive antagonism can be assumed applicable to the data obtained.

It can be seen from the values for $pA_2$ (Xo) that the dibenzocycloheptenes are approximately ten-fold and the dibenzazepines two hundred-fold less powerful antagonists of carbachol than atropine.

## Discussion

The $pA_2$ figure for atropine of 8·58 is in agreement with the value of 8·61 found by Schild (1947) using a guinea-pig ileum preparation with acetylcholine as agonist and a 14 min equilibration with the antagonist. As stated by Arunlakshana & Schild (1959), this relatively short length of time may not, in fact, be long enough for an equilibrium in the atropine concentration to be reached. The problem of uncertain attainment of equilibrium antagonist concentration has been reviewed by Furchgott (1955). The possibility that equilibrium atropine concentration is not reached and that very low atropine concentrations may potentiate rather than antagonize cholinergic agonists, may explain why the results obtained at low concentrations of atropine appeared to fit the competitive antagonism theory under our conditions, whereas concentrations greater than that necessary to produce a 49× change in dose ratio ($8{\cdot}62 \times 10^{-8}$ mol/litre), fit neither the model nor

TABLE 1. *Results of the statistical analysis of the anticholinergic action of atropine and several tricyclic compounds*

| Antagonist | No. of experiments | Ao/A | Slope (b) | $pA_2$ | Regression ($F_R$) | Linearity ($F_D$) | $b \neq -1$ ($t$)* |
|---|---|---|---|---|---|---|---|
| Atropine | 7 | 9–49 | −1·11 | 8·58±0·18 | VHS | NS | NS |
| Dibenzocycloheptenes: | | | | | | | |
| GP 45437 | 14 | 10–169 | −0·97 | 7·95±0·22 | S | NS | NS |
| Amitriptyline | 25 | 5–108 | −1·01 | 7·62±0·26 | VHS | NS | NS |
| Protriptyline | 25 | 3–170 | −0·88 | 7·22±0·08 | VHS | NS | NS |
| Dibenzazepines: | | | | | | | |
| DMI | 23 | 5–102 | −1·10 | 6·38±0·23 | VHS | NS | NS |
| Opipramol | 12 | 13–70 | −1·12 | 5·97±0·37 | S | NS | NS |

$$* t = \frac{b-(-1)}{S_b}$$

the hypothesis used. Failure to attain antagonist equilibrium may also explain why the results obtained with high concentrations of opipramol did not fit the model chosen. However, with an equilibrium period longer than 10 min a diminished tissue response, even in the absence of antagonist, was found. With the other drugs tested the data were found to fit the model based on competitive antagonism theory, over a relatively wide range of antagonist concentration.

In order to overcome any problems arising from the hydrolysis of acetylcholine, carbachol which is not a cholinesterase substrate, but, indeed a cholinesterase inhibitor (Wilson, Hatch & Ginsburg, 1960) was used. However, it has been suggested that carbachol can release acetylcholine from ganglionic presynaptic terminals (Volle & Koelle, 1961) and this release of acetylcholine and inhibition of its breakdown may have been a possible source or variability.

According to Burn & Rand (1965) it is possible that acetylcholine could cause a release of noradrenaline. It is also known that desmethylimipramine and other tricyclic compounds inhibit the uptake of noradrenaline (Iversen, 1965). A part of the apparent antagonism of carbachol produced by these tricyclic compounds could be due to a potentiation of the catecholamine-induced relaxation. However, according to the results of Maxwell, Chaplin, Eckhardt, Soares & Hite (1970), desmethylimipramine is ten-fold more active in inhibition of noradrenaline uptake than protriptyline, whereas for carbachol antagonism, according to the results obtained here, the ratio is substantially reversed.

All calculations are based on the attainment of maximal contraction in the presence of a very high level of agonist after antagonist addition, prior to which the agonist concentration was limited to $0{\cdot}93–1{\cdot}30\times10^{-6}$ mol/litre. This level was found to produce response maxima approximately equal to those obtained after antagonist addition. As stated in the **Results** section the average Ao value for carbachol was $2{\cdot}08\times10^{-7}$ mol/litre, a value comparable to that of approximately $1{\cdot}4\times10^{-7}$ mol/litre calculated from the graphs given by Furchgott & Bursztyn (1967).

Table 2 gives a comparison of the data obtained here with the results of Theobald *et al.* (1965). It can be seen that relative to atropine, the antagonism obtained is of the same order in both sets of results for amitriptyline, DMI and opipramol, except that the values in this report show all three antagonists to be slightly more potent relative to atropine.

TABLE 2. *Comparison of the results reported in this publication* (2) *for the anticholinergic activity relative to atropine, expressed as equiactive doses, of several tricyclic antidepressants with those reported by Theobald,* et al. (1965) (1)

| | (1) | (2) | (2)/(1) |
|---|---|---|---|
| Agonist: | Acetylcholine | Carbamylcholine | — |
| Preparation: | Guinea-pig ileum | Rat fundus | — |
| Antagonist: | | | |
| Dibenzazepines: | | | |
| Opipramol | 305 | 408 | 1·3 |
| DMI | 68 | 158 | 2·3 |
| Dibenzocycloheptene: | | | |
| Amitriptyline | 7 | 9 | 1·3 |
| Atropine | 1 | 1 | 1 |

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