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Factors affecting the binding of tricyclic tranquillizers and antidepressants to human serum albumin

D. SHARPLES

Department of Pharmacy, The University, Manchester M13 9PL, U.K.

The linear free energy-related model for structure activity relations developed by Hansch & Fujita (1964) has been used to correlate the binding of tricyclic tranquillizers and antidepressants to human serum albumin (HSA) with hydrophobic and electronic parameters. The parameters chosen being the chromatographic parameter (R_m) and the affinity of charge transfer complex formation (k_c). The relative importance of these factors has been assessed by linear and multiple linear regression analysis. Results show that the major factor in binding is electronic with only a minor contribution from the hydrophobic parameter.

The nature of the binding site to serum albumin for tricyclic tranquillizers and antidepressants is thought to be hydrophobic (Jähnchen, Krieglstein & Kuschinsky, 1969). The affinity of binding, therefore, ought to be related to the degree of hydrophobicity of the molecule as measured by the log partition coefficient (1pc). Results obtained for the affinity of binding, (Sharples, 1975) indicate that this relation is not quite as close as might be expected, e.g. comparing imipramine ($k = 0{\cdot}24 \times 10^5$, 1pc 2·51) with desipramine ($k = 0{\cdot}7 \times 10^5$, 1pc 1·48), desipramine has a much greater affinity, than its hydrophobicity would indicate. Also, one might have expected that the affinity of promazine ($k = 0{\cdot}85 \times 10^5$, 1pc 2·55), would be similar to that of imipramine. It would appear therefore that more than one factor is involved in determining the serum albumin affinity of this group of compounds.

It was decided to investigate factors affecting the serum albumin binding affinity of these compounds by the linear free energy-related models developed by Hansch & Fujita (1964). These models relate the biological response of a series of compounds to various physical parameters. Two parameters were chosen for this work, a hydrophobic parameter (R_m) and an electronic parameter (log k_c).

R_m is the chromatographic parameter and is defined as $\log(1/R_F - 1)$. It has been shown to be linearly related to the hydrophobic substituent parameter π originally used by Iwasa, Fujita & Hansch (1965).

The electronic parameter chosen was log k_c which can be defined as the log of the affinity of charge transfer complex formation between a donor and an acceptor molecule. It has been shown by Hetnarski & O'Brien (1975) that this affinity is linearly related to σ, the Hammet substituent parameter originally used by Hansch and was chosen both for its ease of measurement and to parallel the proposed involvement of charge transfer complex formation in the tranquillizing action of these compounds (Karreman, Isenberg & Szent-Györgyi, 1959).

The following equations were therefore proposed to relate the serum albumin binding affinity of tricyclic tranquillizers and antidepressants to hydrophobic and electronic parameters.

$$\log K = a_1 R_m + a_o \quad (1)$$
$$\log K = a_1 R_m + a_2 R_m^2 + a_o \quad (2)$$
$$\log K = a_1 \log k_c + a_o \quad (3)$$
$$\log K = a_1 \log k_c + a_2 \log k_c^2 + a_o \quad (4)$$
$$\log K = a_1 \log k_c + a_2 R_m + a_o \quad (5)$$

where a_o, a_1, and a_2 are constants and were determined by linear and multiple (3-variable) regression analysis.

MATERIALS AND METHODS

Materials

Human serum albumin was a lyophilized preparation for transfusion and a gift of the Manchester Blood Bank. Albumin solutions in Sørensen phosphate buffer (pH 7·4) were prepared immediately before use and assayed spectrophotometrically for albumin. The drugs used were gifts of the following companies, May and Baker Ltd. (chlorpromazine hydrochloride, promethazine hydrochloride, prochlorperazine mesitylate and trimipramine maleate), CIBA-Geigy (U.K.) Ltd. (imipramine hydrochloride, chlorimipramine hydrochloride and desipramine hydrochloride), Smith, Kline and French Laboratories Ltd. (trifluperazine hydrochloride and nortriptyline hydrochloride), John Wyeth and Brother Ltd. (promazine hydrochloride) and Merck, Sharp and Dohme Ltd. (amitriptyline hydrochloride). Riboflavin was obtained from Koch-Light Laboratories Ltd.

Methods

Spectrofluorimetric quenching titrations. These were carried out by the method described in an earlier paper (Sharples, 1975), see Table 1.

Table 1. *Serum albumin binding affinity constants (K) for a series of tricyclic tranquillizers and antidepressants.*

	Compound	$K \times 10^5$ litres mol^{-1}	$K \times 10^5$ (calculated) litres mol^{-1}	Log K	Log K (calculated)
1	Prochlorperazine	2·798	3·20	5·4469	5·5053
2	Trifluperazine	2·86*	2·79	5·4564	5·4452
3	Chlorpromazine	1·90*	1·74	5·2788	5·2405
4	Promethazine	0·79*	0·64	4·8976	4·8032
5	Promazine	0·85*	0·98	4·9294	4·9847
6	Imipramine	0·24*	0·29	4·3802	4·4558
7	Trimipramine	0·24*	0·29	4·3802	4·4712
8	Chlorimipramine	0·73*	0·67	4·8633	4·8246
9	Desipramine	0·70*	0·56	4·8451	4·7504
10	Amitriptyline	0·329	0·27	4·5172	4·4360
11	Nortriptyline	0·42	0·48	4·6232	4·6820

* Data from Sharples (1975).

Determination of R_m *values by reverse phase chromatography*

The relative hydrophobicity of the compounds under investigation was characterized by the R_m value which was determined by reverse phase chromatography, (Boyce & Milborrow, 1965). Strips of Eastman Chromagram sheet 13181 Silica Gel (10 cm × 4 cm) were soaked in a 5% v/v solution of liquid paraffin B.P. in ether taking care that the sheets were evenly coated. The ether was allowed to evaporate leaving the strips evenly coated with liquid parafin. Solutions (0·5 μl) of the free bases of the compounds under investigation (5 mg ml^{-1}) were spotted onto the strips in a random order, 4 spots per strip. A total of 12 spots for each compound, i.e. 34 strips, were prepared. The chromatograms were developed using Eastman 'Chromagram' Developing Apparatus Model 104 with acetone–water (9 : 1) as developing solvent. The spots were located by viewing under ultraviolet light. Under these conditions very little spreading of the spots occurred. The mean R_F-value from 12 measurements was determined and the R_m values calculated (Table 2).

Table 2. *Chromatographic parameters for tricyclic tranquillizers and antidepressants*

Compound	R_F	R_m	R_m^2
Prochlorperazine	0·462(±0·045)	+0·066	0·0044
Trifluperazine	0·581(±0·048)	−0·142	0·0201
Chlorpromazine	0·599(±0·044)	−0·174	0·0302
Promethazine	0·68 (±0·06)	−0·327	0·107
Promazine	0·457(±0·041)	+0·075	0·0056
Imipramine	0·453(±0·035)	+0·08	0·0065
Trimipramine	0·784(±0·026)	−0·559	0·0313
Chlorimipramine	0·635(±0·034)	−0·24	0·0574
Desipramine	0·261(±0·041)	+0·453	0·205
Amitriptyline	0·629(±0·029)	−0·23	0·0528
Nortriptyline	0·373(±0·025)	+0·225	0·0508

Determination of the charge transfer complex formation affinity (k_c)

The method used was an adaption of that described by Yagi, Ozawa & Nagatsu, (1959), and depends on the quenching of the fluorescence of a riboflavin acceptor molecule on forming a charge transfer complex with a suitable donor moecule (see also Karreman & others, 1959). A solution of riboflavin (9×10^{-7}M) in Sørensens phosphate buffer (pH 6·5) was used. The excitation and emission wavelengths of riboflavin are 370 and 520 nm respectively. Fluorescence quenching was measured using a Baird-Atomic Fluorispec SF 100 E spectrofluorimeter, using 4 × 0·7 ml cells as follows: cell 1, 0·7 ml phosphate buffer (pH 6·5); cell 2, 0·7 ml riboflavin solution; cell 3, 0·7 ml phosphate buffer (pH 6·5); cell 4, 0·7 ml riboflavin solution.

To cells 3 and 4 were added donor molecule solution at concentrations in the range 0–7·0 × 10^{-4} M in 0·7 × 10^{-4}M stages, a correction being made for volume changes when determining the total donor molecule concentration. The fluorescence of all 4 cells was measured after each addition to compensate for any instrumental variation. The theoretical fluorescence (F_0) assuming no quenching occurs was determined from the reading for cell 2−1 + 3. The reading for cell 4 gives the actual quenched fluorescence (F). The affinity constant for charge transfer complex formation (k_c)

can then be determined by using the Stern-Volmer equation (Stern & Volmer, 1919),

$$F_o/F = 1 + k_c\,(D)$$

where F_o = theoretical fluorescence, F = quenched fluorescence, and (D) = concentration of donor molecule.

Hence a plot of F_o/F against (D) will be linear and k_c can be determined from the gradient, (Table 3).

Table 3. *Charge transfer complexing affinity constants (k_c) for tricyclic tranquillizers and antidepressants.*

Compound	$k_c \times 10^2$ litres mol^{-1}	log k_c	log k_c^2
Prochlorperazine	4·15 (±0·4)	2·6180	6·8539
Trifluperazine	4·05 (±0·33)	2·6075	6·7991
Chlorpromazine	3·32 (±0·28)	2·5211	6·3559
Promethazine	2·20 (±0·32)	2·3428	5·4887
Promazine	2·475(±0·23)	2·3936	5·7293
Imipramine	1·475(±0·13)	2·1614	4·6716
Trimipramine	1·613(±0·09)	2·2161	4·9111
Chlorimipramine	2·193(±0·21)	2·3455	5·5014
Desipramine	1·825(±0·19)	2·2613	5·1135
Amitriptyline	1·50 (±0·17)	2·1761	4·7354
Nortriptyline	1·773(±0·2)	2·2487	5·0567

RESULTS

Linear and multiple regression analysis on the results presented in Tables 1, 2, and 3 yielded equations 6–10.

$$\text{Log K} = 0{\cdot}167\ (\pm 1{\cdot}03)R_m + 4{\cdot}886 \quad (6)$$
$r = 0{\cdot}121$, $s = 0{\cdot}407$, $n = 11$.

$$\text{Log K} = -0{\cdot}005\ (\pm 0{\cdot}0005)R_m - 1{\cdot}753\ (\pm 0{\cdot}021)R_m^2 + 5{\cdot}01 \quad (7)$$
$r = 0{\cdot}439$, $s = 0{\cdot}391$, $n = 11$.

$$\text{Log K} = 2{\cdot}303\ (\pm 0{\cdot}4)\ \log k_c - 0{\cdot}546 \quad (8)$$
$r = 0{\cdot}975$, $s = 0{\cdot}092$, $n = 11$.

$$\text{Log K} = 7{\cdot}163\ (\pm 0{\cdot}147)\ \log k_c - 1{\cdot}015\ (\pm 0{\cdot}1)\ \log k_c^2 - 6{\cdot}4 \quad (9)$$
$r = 0{\cdot}976$, $s = 0{\cdot}095$, $n = 11$.

$$\text{Log K} = 2{\cdot}304\ (\pm 0{\cdot}047)\ \log k_c + 0{\cdot}173\ (\pm 0{\cdot}006)\ R_m - 0{\cdot}538 \quad (10)$$
$r = 0{\cdot}983$, $s = 0{\cdot}08$, $n = 11$.

r = regression coefficient, s = standard error, n = no. of results.

Analysis of these equations reveals that a very poor linear correlation between serum binding affinity and hydrophobicity (eqn 6) which can be improved somewhat by presenting the equation in a parabolic form (eqn 7) but is still not statistically significant. Equations 8 and 9 on the other hand reveal a very good correlation between serum binding affinity and affinity of charge transfer complex formation, no signifi-

cant improvement being obtained by presenting the results in a parabolic form. Combining the two parameters results in equation 10 which shows a slightly improved correlation over equations 8 and 9 suggesting that the main factor influencing the serum binding affinity of this series of compounds is electronic with only a small hydrophobic contribution. Using equation 10 the calculated values for serum binding affinity can be obtained (Table 1) and a plot of calculated against observed affinity is linear with a regression coefficient of 0·988 (Fig 1).

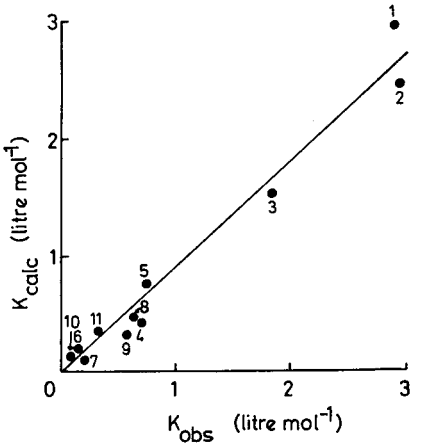

FIG. 1. Plot of observed serum albumin binding affinity against calculated serum albumin binding affinity (numbers refer to Table 1). Regression coefficient r = 0·988, standard error s = 0·166.

DISCUSSION

The importance of electronic factors in the binding of tricyclic tranquillizers and antidepressants to HSA may be explained if one considers the requirements for charge transfer complex formation. It has been established, (Chignell, 1972) that the binding of basic drugs to HSA is primarily an interaction between the aromatic ring system of the drug and the aromatic ring of the tryptophan residue of the serum albumin. Two conditions must be fulfilled, a) steric, there must be very close contact between the π-orbitals of the two aromatic systems and b) electronic, the donor molecule must be sufficiently electron-rich to enable it to donate electrons easily.

Mercier & Dumont (1972) have shown that there is no significant difference between the electron donating ability of the phenothiazine and the iminodibenzyl ring systems thus the observed differences in binding affinity and charge transfer complex formation affinity must be due to steric differences since the relative hydrophobicities of the two groups are also similar. The phenothiazine ring system is virtually planar. Measurements using Dreiding stereo models show that the angle of flexure (α) between two aromatic rings of the phenothiazine system is only 25°. The iminodibenzyl system on the other hand is considerably out of plane, α being 55° (Wilhelm, 1975). Consequently the phenothiazines will be able to approach more closely to both the tryptophan of the serum albumin and the riboflavin model acceptor molecule thus accounting for the observed differences in the two series of drugs.

Differences within the series may be explained by differences in electron richness of the ring system. The presence of an electronegative substituent at position C–2 of the ring system leads to an increase in charge transfer complex formation affinity

and of binding affinity (cf. imipramine and chlorimipramine, promazine and chlorpromazine). An electronegative substituent could have two opposing effects, a negative inductive effect (−I) reducing the electron richness of the ring or a positive conjugative effect (+R) increasing the electron richness of the ring system. A +R effect increasing the electron richness and consequently increasing the electron donating ability of the ring system would explain the observed effects on binding and charge transfer complex formation affinities and also the observed differences in pharmacological potency if activity is related to electron donating ability as is suggested in the phenothiazines (Karreman, & others, 1959).

Nash & Allison, (1963) have suggested that the phenothiazine side chain nitrogen provides an extra binding site to facilitate close approach of the ring system to the acceptor. This may explain the enhanced affinity of desipramine over imipramine, nortriptyline over amitriptyline and prochlorperazine over chlorpromazine since both these compounds have greater capacity for side chain binding (the secondary nitrogen in desipramine and nortriptyline and the second tertiary nitrogen of the piperazino ring of prochlorperazine).

From the results presented, it can therefore be concluded that the major factor influencing binding of tricyclic tranquillizers and antidepressants to HSA is the ability to form a strong charge transfer complex with the tryptophan residue which is itself dependant on steric and electronic factors. Hydrophobicity is only a minor factor.

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