Competition for plasma protein binding sites between phenothiazine tranquillizers and iminodibenzyl antidepressants

Some groups of basic drugs bind to serum albumin e.g. phenothiazines (Jähnchen, Krieglestein & Kuschinsky, 1969), iminodibenzyl antidepressants (Glasser & Krieglestein, 1970) and tetracyclines (Ma, Jun & Luzzi, 1973). Although it is well established that groups of acidic drugs can displace one another from albumin binding sites e.g. aspirin can displace warfarin (Sellers & Koch-Wesser, 1971), little seems to be known about the occurrence of this phenomenon with basic drugs. Such a displacement could have clinical significance when drugs are prescribed together. Consequently the kinetics *in vitro* of binding of a series of iminodibenzyl antidepressants, have been determined in the absence and presence of a series of phenothiazine tranquillizers.

The technique used was that of spectrofluorimetric quenching titration (Chignell, 1972), and the results were evaluated using the Scatchard (1949) method.

Human serum albumin (HSA) was a lyophilized preparation for transfusion and a gift of the Manchester Blood Bank. Albumin solutions in Sorensen phosphate buffer (pH 7·4) were prepared immediately before use and assayed spectrophotometrically for albumin. The drugs used were gifts of May and Baker Ltd. (chlorpromazine hydrochloride, promethazine hydrochloride, trimipramine maleate), CIBA-Geigy (UK) Ltd. (imipramine hydrochloride, chlorimipramine hydrochloride, desipramine hydrochloride), Smith, Kline and French Laboratories Ltd. (trifluperazine hydrochloride) and John Wyeth and Brother Ltd. (promazine hydrochloride).

The titrations were made using a Baird-Atomic Fluorispec SF 100 E spectrofluorimeter. The excitation and emission wavelengths of HSA are 285 and 354 nm respectively and iminodibenzyl drug concentrations in the range 0–70 $\times$ 10^{-6}M in 7·143 $\times$ 10^{-6}M stages were titrated against HSA solution (5–6 $\times$ 10^{-6}M in Sorensen phosphate buffer pH 7·4). The titrations were then repeated in the presence of phenothiazine (concentration 14·285 $\times$ 10^{-6}M). Finally the phenothiazines alone were titrated at concentrations in the range 0–20 $\times$ 10^{-6}M in 0·7143 $\times$ 10^{-6}M stages. The total volume of HSA solution titrated was 0·7 ml and a correction was made for volume changes when determining the total drug concentration. Measurements were made at room temperature (20°).

The calculated values for the number of binding sites (n) and the affinity of binding (k) for iminodibenzyl antidepressants in the absence and presence of phenothiazines and for phenothiazines alone are given in Tables 1 and 2.

It has been previously demonstrated, (Sharples, 1974) that spectrofluorimetric quenching titration gives results directly comparable to those obtained by equilibrium dialysis and so give a true measure of the kinetics of binding.

The Scatchard equation defining the kinetics of protein binding can be stated as follows: $r/D_F = kn - kr$ where r = moles drug bound mole^{-1} protein; D_F = moles drug free; n = no. of binding sites mole^{-1} protein; k = affinity of binding.

In the presence of a successful competitor, drug r will decrease and consequently D_F will increase. Therefore to maintain the balance of the equation either k or n must decrease. If the competition is reversible the total number of binding sites will remain constant and competition will result in a reduced affinity of the drug for the protein. If, however, competition is not reversible the affinity of the drug will remain the same but the total number of binding sites will be greatly reduced.

The results in Table 1 indicate that the number of binding sites for iminodibenzyl antidepressants in 1–2 mole^{-1} protein. The number is not significantly altered by the

Table 1. *Binding constants for the binding of iminodibenzyl antidepressants in the absence and presence of phenothiazine tranquillizers.*

Drug combination (concentrations M)	n	K(litres mol^{-1})
Imipramine alone	1·31	$0·239 \times 10^5$
+ promazine ($14·285 \times 10^{-6}$)	1·03	$0·159 \times 10^5$
+ promethazine ($14·285 \times 10^{-6}$)	1·65	$0·131 \times 10^5$
+ chlorpromazine ($14·285 \times 10^{-6}$)	1·402	$0·074 \times 10^5$
+ trifluperazine ($14·285 \times 10^{-6}$)	1·436	$0·063 \times 10^5$
(HSA concentration $6·087 \times 10^{-6}$)		
Trimipramine alone	1·24	$0·238 \times 10^5$
+ promazine ($14·285 \times 10^{-6}$)	1·3	$0·158 \times 10^5$
+ promethazine ($14·285 \times 10^{-6}$)	1·28	$0·133 \times 10^5$
+ chlorpromazine ($14·285 \times 10^{-6}$)	1·47	$0·06 \times 10^5$
+ trifluperazine ($14·285 \times 10^{-6}$)	1·3	$0·07 \times 10^5$
(HSA concentration $6·087 \times 10^{-6}$)		
Chlorimipramine alone	1·03	$0·735 \times 10^5$
+ promazine ($14·285 \times 10^{-6}$)	1·11	$0·406 \times 10^5$
+ promethazine ($14·285 \times 10^{-6}$)	1·03	$0·407 \times 10^5$
+ chlorpromazine ($14·285 \times 10^{-6}$)	1·19	$0·302 \times 10^5$
+ trifluperazine ($14·285 \times 10^{-6}$)	1·3	$0·245 \times 10^5$
(HSA concentration $5·362 \times 10^{-6}$)		
Desipramine alone	1·53	$0·702 \times 10^5$
+ promazine ($14·285 \times 10^{-6}$)	1·5	$0·368 \times 10^5$
+ promethazine ($14·285 \times 10^{-6}$)	1·6	$0·376 \times 10^5$
+ chlorpromazine ($14·285 \times 10^{-6}$)	1·2	$0·304 \times 10^5$
+ trifluperazine ($14·285 \times 10^{-6}$)	1·11	$0·16 \times 10^5$
(HSA concentration $5·797 \times 10^{-6}$)		

Table 2. *Binding constants for the binding of phenothiazine tranquillizers.*

Phenothiazine	HSA Concentration (M)	n	K(litres mol^{-1})
*Chlorpromazine	$6·8 \times 10^{-6}$	1·94	$1·9 \times 10^5$
Promazine	$5·797 \times 10^{-6}$	1·31	$0·85 \times 10^5$
Promethazine	$5·797 \times 10^{-6}$	1·64	$0·79 \times 10^5$
Trifluperazine	$5·797 \times 10^{-6}$	1·18	$2·86 \times 10^5$

* Data from Sharples (1974).

presence of phenothiazine tranquillizers. The affinity of binding however is greatly reduced by the presence of phenothiazine tranquillizers. Phenothiazines do therefore displace iminodibenzyl antidepressants from protein binding sites *in vitro* and the competition is reversible. Phenothiazines displace iminodibenzyl compounds and not vice versa since the affinities of the phenothiazines are always greater (Tables 1 and 2). The extent to which displacement occurs seems therefore to be related to the relative affinities of the two compounds concerned. The greater the difference between the two affinities, the greater will be the amount of displacement.

The concentrations used in the present work (0–19·6 μg ml^{-1} desipramine competing against 5 μg ml^{-1} chlorpromazine, for example) are much higher than the reported plasma concentrations of desipramine (0·033–0·29 μg ml^{-1}, Borgå, Azarnoff & others, 1969) and of chlorpromazine (0·024–0·477 μg ml^{-1}, Curry, 1968). Also, a purified serum albumin preparation was used instead of plasma so these *in vitro* findings cannot be directly related to *in vivo* situation. However these *in vitro* findings may throw light on the protein binding site for the drugs.

The nature of the binding site for phenothiazines and iminodibenzyl compounds is assumed to be hydrophobic, (Jähnchen & others, 1969). The affinity of binding, therefore, ought to be related to the degree of hydrophobicity of the molecule as measured by the partition coefficient. On the whole, the results presented appear to substantiate this assumption. The position of desipramine, however, is anomalous. For chlorimipramine $k = 0{\cdot}73 \times 10^5$ and the log partition coefficient (lpc) (Glasser & Krieglestein, 1970) = 3·32; for imipramine $k = 0{\cdot}24 \times 10^5$ and lpc = 2·51 while for desipramine $k = 0{\cdot}7 \times 10^5$ and lpc = 1·48.

Desipramine has a far greater affinity than its partition coefficient would suggest. This would indicate that other factors are involved in determining the affinity of binding of these compounds, possibly ionic since desipramine is much more basic than any of the other compounds (desipramine pka = 10·2, imipramine pka = 9·5, Green, 1967).

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

D. SHARPLES

November 20, 1974

REFERENCES

BORGÅ, O., AZARNOFF, D. L., FORSHELL, G. P. & SJÖQVIST, F. (1969). *Biochem. Pharmac.*, **18**, 2135–2143.

CHIGNELL, C. F. (1972). In *Methods in Pharmacology* vol. 2 Physical Methods p.33. New York: Meredith.

CURRY, S. H. (1968). *Analyt. Chem.*, **40**, 1251–1255.

GLASSER, H. & KRIEGLESTEIN, J. (1970). *Arch. Pharm. exp. Path.*, **265**, 321–334.

GREEN, A. L. (1967). *J. Pharm. Pharmac.*, **19**, 10–16.

JÄHNCHEN, E., KRIEGLESTEIN, J. & KUSCHINSKY, G. (1969). *Arch Pharm. exp. Path.*, **263**, 375–386.

MA, J. K. H., JUN, H. W. & LUZZI, L. A. (1973). *J. pharm. Sci.*, **62**, 1261–1264.

SCATCHARD, G. (1949). *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

SELLERS, E. M. & KOCH-WESSER, J. (1971). *Ibid.*, **179**, 213–225.

SHARPLES, D. (1974). *J. Pharm. Pharmac.*, **26**, 640–641.

The antipseudomonal activity of contact lens solutions

The antimicrobial effectiveness of contact lens solutions have been reported (Norton, Davies & others 1974; McBride & Mackie, 1974). My own evaluations of the antibacterial efficiency of these solutions support the proposition made by the above workers that suitable standards should be applied to contact lens solutions. An important part of any standards should include standard methods of evaluation. Uniform methods for the preparation of test inocula of microorganisms need to be included because when the findings of Norton & others (1974) using *Pseudomonas aeruginosa* NCTC 6750 are compared (see Table 1) with a similar evaluation using the same test organism and the same contact lens solutions, but using the technique of Richards & McBride (1971), several of the findings are markedly different. The difference in the size of the inocula 10^6 organisms ml^{-1} used by Norton & others compared with 5×10^6 organisms ml^{-1} in this present investigation is not sufficient to account for the difference in the results reported. Neither does it appear that the differences in results can arise from changes occuring in the commercially prepared solutions during storage, since the results obtained were similar for the commercial preparations and for the equivalent freshly prepared preparations.

Norton & others (1974) washed the cell suspensions, used as sources of inocula, with a minimal salts medium. With *P. aeruginosa* this could have affected the subsequent resistance of the cells to chemical inactivation and their results would seem