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Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components

M. H. BICKEL

Medizinisch-chemisches Institut, University of Berne, Berne, Switzerland

The binding of model drugs to human blood and individual blood components has been determined by equilibrium dialysis and expressed in terms of classes of binding sites, association constants and binding capacities. Chlorpromazine and imipramine are bound to three major components: membranes of red cells, albumin, and lipoproteins. The affinity and capacity of lipoprotein binding is at least as high as that of albumin and is equally distributed on HDL, LDL, VLDL, and on the chylomicrons. White blood cells and platelets are of minor importance in terms of binding capacity. No binding was detected with γ-globulins or α- and β-globulins other than lipoproteins. In contrast, salicylic acid was not bound to red cells or lipoproteins.

Only recently have binding sites for drugs in blood other than albumin received attention, e.g., globulins (Meyer & Guttman, 1968; Dayton, Israili & Perel, 1973) or red blood cell uptake of chlorpromazine (Freeman & Spirtes, 1963; Ahtee & Paasonen, 1966; Jähnchen, Krieglstein & others, 1971; Manian, Piette & others, 1974) and other drugs (McArthur, Dawkins & Smith, 1971). Studies aimed at identification of all the binding sites in blood for one drug are still lacking but would be useful since such a study could serve as a model for the more complex situation in tissues and the pharmacokinetics of a drug can only be fully characterized if both sets of binding sites are known. This widened view of drug binding to non-specific receptors and its pharmacokinetic consequences has been discussed by Gillette (1973), Marks, Lindup & Baylis (1973) and Dayton & others (1973).

We have attempted to detect and characterize binding sites in blood components other than albumin for two basic lipophilic drugs, chlorpromazine and imipramine, and a representative of acidic drugs, salicylic acid. These three drugs, with others, have been used in a previous study on tissue binding (Bickel & Steele, 1974) and thus allow to some degree a comparison between intracellular and blood binding.

MATERIALS AND METHODS

Blood fractions

Red blood cells (RBC) and plasma were separated by centrifugation of citrated human blood. For the preparation of membrane-free RBC contents the cell sediment was washed three times with NaCl 0·9%, haemolysed by adding an equal amount of water, followed by freezing, ultracentrifugation for 1 h at 100 000 *g* and ultrafiltration with a Millipore 45 μm. RBC membranes were prepared from washed cells by suspension in phosphate buffer 310 mosm, pH 8, haemolysis in the same buffer at

10 mosm, centrifugation of the ghosts at 30 000 *g*, washing three times and resuspension in phosphate buffer 310 mosm, pH 7·4.

The lipoprotein fractions HDL, LDL, and VLDL as well as the total lipoprotein and chylomicrons were prepared from plasma by the flotation method in density media in a preparative ultracentrifuge as indicated by Lindgren, Nichols & Wills (1961) as modified by Hatch & Lees (1966). Since the normal plasma samples contained negligible amounts of chylomicrons, this fraction was prepared from a hyperlipaemic plasma of a total lipid content of 1305 mg per 100 ml. Binding values of salicylic acid to lipoprotein and diluted human serum albumin (HSA) solutions indicated that the total lipoprotein fractions contained less than 1% of the plasma albumin. The lipoprotein-free globulin fraction was prepared from the lipoprotein-free infranatant of the plasma, precipitation of globulins with ammonium sulphate at half saturation and dialysis of the precipitate against NaCl 0·9%. The cholesterol content of this fraction was 11 $\pm$ 1 mg per 100 ml, corresponding to less than 5% of the plasma cholesterol.

All these fractions prepared from whole blood were rediluted with NaCl 0·9% to their original concentrations in whole blood. Human white blood cell and platelet preparations were obtained from the Theodor Kocher Institute of the University of Berne. They were resuspended in NaCl 0·9% according to their counts to correspond to their concentration in whole blood. Human serum albumin and human γ-globulin were obtained from the Central Laboratory of the Swiss Red Cross in Berne. They were dissolved in NaCl 0·9% and used at concentrations of 2·0 and 0·5 g per 100 ml according to their approximate concentrations in whole blood at a haematocrit value of 0·5. The average values of cholesterol and total lipid in the plasma samples used were 228 $\pm$ 16 and 925 $\pm$ 76 mg per 100 ml respectively.

Binding experiments

Equilibrium dialysis was as described by Bickel & Steele (1974) with a DIANORM apparatus. 1 ml of blood or blood fraction was dialysed against 1 ml of NaCl 0·9% for 2·5 h at 37°. The drugs were added to the blood or blood fraction at concentrations from 40 to 1600 μM (salicylic acid 10 to 4000 μM). Equilibrium concentrations of the drugs were determined in the saline cell and, in the absence of haemoglobin, in the protein containing cell. The binding affinities and capacities for the individual binding sites were calculated by Scatchard analysis (Bickel & Steele, 1974).

Drugs and drug analyses

Chlorpromazine hydrochloride was obtained from the Psychopharmacology Service Center, NIMH, Rockville, MD, and determined spectrophotometrically at 255 nm in aqueous solution. Imipramine hydrochloride (Ciba-Geigy, Basel) was determined fluorimetrically at 294/415 nm as described by Bickel & Weder (1969). Comparable results were obtained with 10-[^{14}C]imipramine in a few experiments. This drug as well as salicylic acid [^{14}C]carboxyl were purchased from The Radiochemical Centre, Amersham, U.K. They were determined by liquid scintillation counting.

RESULTS

The binding of the three model drugs to human whole blood and to various components and fractions of blood has been measured and expressed in terms of

overall affinity (association constants K_1) and binding capacities C_1 for each class of binding sites established by Scatchard analysis of the experimental data.

Table 1 contains the binding parameters for chlorpromazine which shows three major binding components: erythrocyte membranes, lipoproteins, and serum albumin. All of the major lipoprotein fractions have comparable affinity (M^{-1}) and capacity values (μmol ml^{-1} blood): HDL $2{\cdot}5 \times 10^3$, 0·7; LDL $2{\cdot}1 \times 10^3$, 0·5; VLDL $1{\cdot}9 \times 10^3$, 0·5. Thus the three fractions contribute evenly to the overall lipoprotein binding.

Table 1. *Binding of chlorpromazine to human blood and blood components. Affinities (K) and capacities (C) in* M^{-1} *and* μ*mol* ml^{-1} *blood respectively (Mean values and range).*

	n	K_1	K_2	C_1	C_{1+2}
Whole blood	3	$1{\cdot}4 \times 10^4$ (1·1–1·6)	$1{\cdot}4 \times 10^3$ (1·0–1·8)	0·9 (0·8–1·0)	4·2 (3·9–4·7)
RBC	3	$8{\cdot}3 \times 10^3$ (7·0–9·4)	$8{\cdot}7 \times 10^3$ (6·7–11)	0·6 (0·5–0·8)	3·2 (2·6–3·7)
RBC membranes	5	$1{\cdot}5 \times 10^4$ (1·0–2·6)	$2{\cdot}7 \times 10^3$ (1·3–4·1)	0·2 (0·1–0·2)	0·5 (0·4–0·6)
RBC contents*	3	$5{\cdot}0 \times 10^3$ (4·0–6·0)			
RBC „ + membr.	3	$2{\cdot}0 \times 10^4$ (1·3–2·9)	$2{\cdot}0 \times 10^3$ (0·9–3·1)	0·3 (0·2–0·4)	1·6 (1·1–2·5)
WBC	2	$7{\cdot}1 \times 10^3$ (5·7–8·4)	$9{\cdot}6 \times 10^3$ (9·5–9·6)	0·04	0·1
Platelets	2	$9{\cdot}7 \times 10^3$ (9·4–10)		0·03	
Plasma	3	$1{\cdot}5 \times 10^4$ (1·2–1·9)	$1{\cdot}4 \times 10^3$ (1·2–1·7)	0·6 (0·5–0·7)	2·6 (2·4–2·8)
HSA	4	$1{\cdot}3 \times 10^3$ (0·8–1·8)		0·9 (0·6–1·2)	
Total LP	5	$3{\cdot}4 \times 10^3$ (2·0–4·6)	$9{\cdot}5 \times 10^3$ (5·8–13)	1·1 (0·8–1·4)	—
LP-free globulin	4	negligible			
HGG	3	negligible			
chylomicrons**	3	$1{\cdot}6 \times 10^3$ (0·6–2·2)		0·5 (0·4–0·7)	

* Membrane-free.
** From hyperlipemic plasma.
RBC Red blood cells. WBC White blood cells. HSA Human serum albumin (commercial). LP Lipoproteins. HGG Human gamma globulin (commercial). n = number of experiments.

An albumin preparation obtained from the plasma samples by ammonium sulphate precipitation showed binding parameters that were comparable to the ones obtained with commercial HSA. Similarly, the binding parameters of a precipitated globulin fraction were comparable to the ones of total lipoproteins.

Tables 2 and 3 represent the binding parameters obtained with the model drugs imipramine and salicylic acid respectively. The ranges of the individual values determined are comparable to the ones given in Table 1. Whereas imipramine has

Table 2. *Binding of imipramine to human blood and blood components. Affinities (K) and capacities (C) in* M^{-1} *and* μ*mol* ml^{-1} *blood respectively.*

	n	K_1	K_2	C_1	C_{1+2}
Whole blood	3	$1{\cdot}4 \times 10^4$	$7{\cdot}1 \times 10^2$	0·3	2·2
RBC	3	$9{\cdot}4 \times 10^3$	$9{\cdot}8 \times 10^2$	0·2	1·2
RBC membranes	8	$1{\cdot}3 \times 10^4$	$1{\cdot}3 \times 10^3$	0·08	0·4
RBC contents*	2	negligible			
WBC	1	$1{\cdot}5 \times 10^3$		0·05	
Platelets	1	$6{\cdot}8 \times 10^2$		0·08	
Plasma	3	$1{\cdot}0 \times 10^4$	$9{\cdot}1 \times 10^2$	0·2	1·0
HSA	7	$4{\cdot}9 \times 10^2$		0·6	
Total LP	3	$2{\cdot}3 \times 10^3$	$5{\cdot}6 \times 10^2$	0·3	1·0
LP-free globulin	2	negligible			
HGG	1	negligible			

* Membrane-free. n=number of experiments. Abbreviations see Table 1.

Table 3. *Binding of salicylic acid to human blood and blood components. Affinities (K) and capacities (C) in* M^{-1} *and* $\mu mol\ ml^{-1}$ *blood respectively.*

	n	K_1	K_2	C_1	C_{1+2}
Whole blood	3	$1{\cdot}0 \times 10^4$	$1{\cdot}3 \times 10^3$	0·4	1·3
RBC	3	negligible			
RBC membranes ..	4	negligible			
Plasma	4	$8{\cdot}2 \times 10^3$	$1{\cdot}2 \times 10^3$	0·4	0·9
HSA	4	$1{\cdot}1 \times 10^4$	$1{\cdot}2 \times 10^3$	0·4	0·9
Total LP	3	negligible			

Abbreviations see Table 1.
n = number of experiments.

the same qualitative binding pattern as chlorpromazine, salicylic acid does not bind to red cell membranes or lipoproteins. Table 4 further characterizes the binding patterns of the three model drugs by giving the fractions bound and free for the subsaturation concentration of 10^{-4} M.

DISCUSSION

As shown in Table 1 the three main binding sites for chlorpromazine are red cells, albumin and lipoproteins. Red cell binding confirms observations reported by Freeman & Spirtes (1963), Ahtee & Paasonen (1966), Jähnchen & others (1971) and Manian & others (1974) although the latter authors report an association constant which is ten times larger. Binding of chlorpromazine to red cells represents mainly binding to its membrane. The association constant for chlorpromazine to human serum albumin is ten times less than to red cells. Other authors (Krieglstein & Kuschinsky, 1969; Nambu & Nagai, 1972; Huang & Gabay, 1974; Sharples, 1974) reported higher values. However, their experimental conditions were different and a valid comparison may not be possible. The binding to the lipoproteins has not been previously described and is evenly distributed amongst the HDL-, LDL-, and VLDL-fractions. The affinities and total capacity tend to surpass the corresponding values for albumin. Chylomicrons, if present, also bind chlorpromazine with similar binding parameters. This is reminiscent of the effect of an infused fat emulsion on plasma binding for this drug, as has been reported by Krieglstein, Meffert & Niemeyer (1974). White blood cells and platelets also bind chlorpromazine. In contrast, no binding to globulins other than lipoproteins including γ-globulins could be detected.

Table 4. *Binding of chlorpromazine, imipramine and salicylic acid to blood and blood components. Percentage bound* (β) *and free* (α) *at* 10^{-4} M.

	Chlorpromazine		Imipramine		Salicylic acid	
	β	α	β	α	β	α
Whole blood	92·7	7·3	76·8	23·2	76·3	23·7
RBC	82·0	18·0	61·3	38·7	<10	>90
Plasma	88·6	11·4	50·3	49·7	72·2	27·8
HSA	46·2	53·8	18·9	81·1	74·9	25·1
Total LP	75·7	24·3	35·3	64·7	1	99

Abbreviations see Table 1.
Concentrations of blood components corresponding to whole blood.

A similar situation is observed with the related drug, imipramine, which also binds to red cells (Bickel & Börner, 1974); this is definitely a binding to the cell membrane, since no binding to erythrocyte contents can be detected. With chlorpromazine, haemoglobin catalyses the formation of chlorpromazine-sulphoxide (Minder, Schnetzer & Bickel, 1971) which may lead to a trapping effect, interfering with the binding experiments. Imipramine, therefore, seems to be a better model in this respect. It also binds to lipoproteins and both affinity and capacity are higher than the corresponding values for albumin. It is therefore suggested that more attention should be paid to the binding to lipoproteins. These binding sites are likely to be important with lipophilic drugs able to show hydrophobic interactions and to represent the binding of drugs to α- and β-globulins occasionally reported. Only rarely has binding to lipoproteins been specifically stated (Rudman, Hollins & others, 1972; Ghosh, Basu & Schweppe, 1974; Powis, 1974). Imipramine also binds to white cells and platelets with relatively high affinity. However, as with chlorpromazine, the binding capacity per volume blood is only a negligible fraction of the capacity of the total red cells. Platelet binding of tricyclic drugs has also been reported by Gaut (1973). With both chlorpromazine and imipramine the sum of the binding capacities of lipoproteins and albumin roughly equals the plasma capacity, and the sum of plasma and red cell is comparable to the capacity of whole blood. This, and the results displayed in Tables 1 and 2, does not necessarily mean that additional binding sites are not present. The fact that with both drugs the association constants determined with total plasma are higher than the corresponding values for lipoprotein and for albumin may even suggest the existence of an unidentified high affinity-low capacity binding site in plasma.

In contrast to these basic lipophilic drugs, salicylic acid has a totally different binding pattern in blood (Table 3). There is no binding to red cells and lipoproteins, and the association constants and capacity values of the two classes of binding sites are identical with whole blood, plasma, and albumin. Thus, albumin is likely to be the only main site for the binding of salicylic acid in blood. The binding parameters are comparable with those recently determined with equilibrium dialysis and other methods by Keresztes-Nagy, Mais & others (1972) and Zaroslinski, Keresztes-Nagy & others (1974).

The numerous blood components able to bind drugs like chlorpromazine or imipramine would be expected to lead to a competition for the drug and this is reflected in Table 4. The fraction bound to one component is dependent on the presence and amount of other binding components. This was also shown in a simple distribution experiment with imipramine whose red cell/medium concentration ratio was 4·0 if the medium was buffer, but decreased to 1·0 if the medium was plasma, i.e. when lipoproteins and albumin competed with the red cell membranes. Such competitive phenomena have also been indicated by Jähnchen & others (1971). For the same reason, the similar percentages bound with plasma and with albumin alone, do not justify considering albumin as the sole binding site in plasma as was done by Weder & Bickel (1970).

In conclusion, the binding of certain classes of drugs to red cells and lipoproteins is likely to be of equal or greater importance than their binding to serum albumin. Pharmacokinetically, the competition of a drug for binding sites between intravascular and intracellular sites may even be more important than the competition within blood. It may therefore be of particular interest to compare binding and binding sites in

blood and in tissues. Whereas salicylic acid is bound to serum albumin but not to intracellular fractions, chlorpromazine and imipramine are bound to several blood components in addition to tissue structures like intracellular membranes (Bickel & Steele, 1974). Interestingly, the binding parameters of these drugs with microsomal or mitochondrial membranes are comparable to those with red cell membranes. The binding parameters reported in these studies may therefore allow a better understanding of how precisely extra- and intracellular binding influences the distribution and other pharmacokinetic properties of a drug.

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