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CHLORPHENTERMINE BINDING IN RAT LUNG SUBCELLULAR FRACTIONS AND ITS DISPLACEMENT BY DESMETHYLIMIPRAMINE

RODNEY F. MINCHIN, KENNETH F. ILETT* and BARRY W. MADSEN

Department of Pharmacology, University of Western Australia, Nedlands, Western Australia, 6009

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Abstract—An examination of possible mechanisms of binding interactions between drugs accumulated in the lung has been made by equilibrium dialysis using [^{14}C]chlorphentermine (CP) in rat lung subcellular fractions (microsomal and 15,000 *g* and cytosol). By far the greatest CP binding was in the microsomal and 15,000 *g* fractions and with all isotherms, CP binding was shown to consist of a specific, saturable component and a non-specific partitioning component. Desmethylimipramine (DMI) was the most potent inhibitor of a series of compounds and was selected for detailed study. Generally, as DMI concentration was increased, CP binding decreased, however this relationship was not in accordance with simple direct competitive theory. At 5×10^{-4} M DMI in the microsomal fraction, CP binding was initially increased with increasing CP concentration. In the 15,000 *g* fraction, the apparent CP affinity constant did not decrease regularly with increasing DMI concentration, the affinity constant of DMI increased with increasing DMI concentration, a Hill coefficient significantly less than unity was obtained and the binding model became increasingly untenable for higher DMI levels. It is likely that DMI and CP interact in rat lung via a co-operative mechanism involving binding-induced conformational transitions. The possible role of phospholipids in the binding of basic amines in lung is discussed.

In recent years there has been a growing awareness of the non-respiratory functions of lung tissue. It is capable of the preferential accumulation of a number of endogenous substances [1] and drugs [2] as well as the biotransformation of some of these compounds [3]. The ability to accumulate and reversibly bind drugs makes the lung a potentially important pharmacokinetic compartment and it is possible that drug interactions comparable to those occurring with plasma proteins may occur in this organ. Several interactions have previously been demonstrated in isolated perfused lung preparations where a decreased accumulation of one drug in the presence of another has been reported [4–6].

Many of the compounds which are selectively distributed to lung tissue have two features in common: (1) they are basic and (2) they have an amine group separated from a hydrophobic ring structure. It has been suggested that these basic amines share common binding sites within the lung and that phospholipids, which occur in high concentration in lung, may be involved [7]. Chlorphentermine (CP), a commonly used anorexic agent, has been shown to accumulate in tissues which have either high content or rapid turnover of phospholipids [8]. Chronic administration of the drug induces pulmonary lipidosis [9] mainly due to decreased degradation of phosphatidylcholine [10]. In the present study we have investigated CP binding to various rat lung subcellular fractions in the presence and absence of a number of compounds, many of which are known to accumulate in the lung. The effect of desmethylimipramine (DMI) on CP binding has been studied in greater detail since it has been shown to inhibit the accumulation of many compounds including 5-hydroxytryptamine [1], metaraminol [11], propranolol [6], imipramine [4, 5] and amphetamine [5] *in vitro* as well as methadone *in vivo* [12].

MATERIALS AND METHODS

Chemicals: 2-[^{14}C]chlorphentermine HCl (specific activity = 5.72 mCi · mmole^{-1}) was obtained from New England Nuclear, USA. The radiopurity was greater than 98 per cent as determined by thin layer chromatography in the following systems: (1) chloroform:methanol (50:50), (2) chloroform:methanol: ammonia (10:90:0.5) and (3) ethanol:benzene:dioxane: ammonia (50:50:40:1). The following compounds were also used: amitriptyline HCl (Merck, Sharp & Dohme, Aust.), *d*-amphetamine SO_4 and caffeine (B.P.), chlorcyclizine HCl (Burroughs Wellcome, Aust.), chloroquine PO_4 (B.P.), chlorphentermine HCl (Aldrich Chemicals, USA), desmethylimipramine HCl (Ciba–Geigy, Aust.), diphenhydramine HCl (Parke Davis & Co., USA), 5-hydroxytryptamine (Calbiochem, Aust.), imipramine HCl (Ciba-Geigy, Aust.), lignocaine HCl (B.P.), mepyramine maleate (May & Baker, Aust.), methadone HCl (Burroughs Wellcome, Aust.), naloxone HCl (Endo Labs., USA), paracetamol (B.P.), paraquat (K & K Labs., USA), pronethalol HCl and propranolol HCl (I.C.I., Aust.), *d*-propoxyphene HCl (Lilly, Aust.), quinidine SO_4 and salicylic acid (B.P.).

Preparation of lung subcellular fractions. Male Wistar rats (180–220 g) were killed by cervical dislocation and lungs were perfused with 20 ml ice cold buffer (0.154 M KCl, 0.05 M phosphate, pH = 7.4) via the pulmonary artery, then removed and homogenised in 5 volumes of buffer at 4° using an Ultra Turrax blender. The subcellular fractions were: (1) the nuclear-cell debris-mitochondrial fraction obtained by centrifuging whole homogenates at 15,000 *g* for 15 min, (2) the microsomal fraction obtained by centrifuging the

* All correspondence to be addressed to Dr. K. F. Ilett, Department of Pharmacology, University of Western Australia, Nedlands, Western Australia, 6009.

supernatant from the previous fraction at 240,000 *g* for 30 min, and (3) the cytosol or microsomal supernatant. Protein concentrations of each fraction, estimated by the method of Hartree [13], were 36.1 ± 1.9, 13.1 ± 1.7, and 42.1 ± 1.2 mg · g^{-1} lung respectively (mean ± S.E.M., $n = 5$). The 15,000 *g* and 240,000 *g* pellets were washed once in buffer, re-sedimented, then resuspended in buffer using an all-glass homogeniser. Each fraction was then adjusted to give a final protein concentration of 2 mg · ml^{-1}.

Binding experiments. The binding of [^{14}C]-CP to subcellular fractions was examined by equilibrium dialysis at 37° using a 1 ml Dianorm apparatus (MSE Scientific Instruments, England) and Spectrapor dialysis membranes (Spectrum Medical Industries Inc., USA) with a molecular weight permeability limit of 12,000–14,000. The subcellular fraction (1 ml) was placed in one half of each cell and CP (2.5×10^{-7}–2×10^{-2} M) in the other. Complete equilibration was shown to occur within 3 hr and the addition of CP initially to either the protein or protein-free side of the cells did not significantly alter the final levels of bound and free drug, indicating that thermodynamic equilibrium was achieved in this time. Binding of CP to the membrane was found to be 6.6 ± 1.2 per cent over the entire concentration range used and total CP concentration was adjusted accordingly. In the interaction experiments, the same concentration of inhibitor was added to each side of the dialysis cell. After equilibration, CP concentration on each side of the cell was assayed by adding a 0.6–0.9 ml aliquot of the dialysate (solubilised with 2 ml "Protosol" (New England Nuclear):isopropanol (1:1) where appropriate) to 10 ml of scintillant cocktail (6 g PPO, 330 ml Brydet X10, 670 ml toluene) and counting ^{14}C in a Hewlett Packard (Model B2450) liquid scintillation counter. Using the thin layer chromatography systems described above, no metabolic transformation of CP was detectable after dialysis for three hr in any of the fractions used.

Data analysis. The concentration of bound CP (C_b) was calculated by substracting the concentration of free CP (C_f = the concentration of CP in the protein-free side of the cells) from the total concentration (C_t) in the protein side. Percent inhibition of CP binding by inhibitor was calculated as:

$$I = 100(C_b - C'_b)/C_b \qquad (1)$$

where C_b = bound concentration of CP in the absence of inhibitor and C'_b = bound concentration of CP in the presence of inhibitor. The apparent affinity constants (K_a) and the binding capacities of different subcellular fractions (n) for CP in the presence of varying levels of DMI were estimated using a non-linear least-squares regression of C_b on C_f [14] assuming the standard model derived from the law of mass action with provision for non-specific partitioning:

$$C_b = nPK_aC_f/(1 + K_aC_f) + K_{0/w}C_f \qquad (2)$$

where P = protein concentration and $K_{0/w}$ is the oil–water partition coefficient of CP. Data were weighted with the reciprocal of their variance ($W_i = 1/\sigma_i^2$). Affinity constants of CP and DMI in the 15,000 *g* fraction were simultaneously estimated from the data assuming (1) CP binding could be described by one class of binding sites plus a non-specific partitioning factor and (2) displacement of CP by DMI was by simple competition for the same binding sites. With these assumptions, bound CP can be estimated by:

$$C_b = nPK_aC_f/(1 + K_aC_f + K_dC_d) + K_{0/w}C_f \qquad (3)$$

where K_d = affinity constant of DMI and C_d free concentration of DMI. C_d can be described as a function of C_f and C_a, the total concentration of DMI added to the cells, by [15]:

$$C_d = \frac{\sqrt{(2 + 2K_aC_f + nPK_d - K_dC_a)^2 + 8K_dC_a \times (1 + K_aC_f)}}{4K_d} \qquad (4)$$

Hill plots were analysed for linearity by stepwise polynomial regression and all comparisons of mean data were done using a one-way analysis of variance or a two-tailed t test.

RESULTS

Figure 1 shows that the three subcellular fractions bound CP to varying extents, being greatest in the microsomal fraction and least in the cytosol fraction. The percent CP bound remained relatively constant in all three fractions up to a total concentration (C_t) of 100 μM; above this level it decreased steadily, indicating the involvement of specific binding rather than a simple partitioning process. At most C_t levels, the concentration of CP bound in the microsomal fraction was one and a half times that in the 15,000 *g* fraction. Since the 15,000 *g* fraction contains three times more protein per gram of lung compared to the microsomal fraction, overall it will probably contribute to a greater extent to CP binding in intact cells and hence it was chosen for detailed study.

A number of compounds were tested as inhibitors of CP binding (5×10^{-7} M) in the 15,000 *g* fraction (Table 1). At 5×10^{-4} M concentration, DMI, amitriptyline, quinidine, chlorcyclizine and imipramine caused more than a 50 per cent decrease in the concentration of bound CP whereas salicylic acid, paracetamol, pentobarbitone and caffeine caused no appreciable change and paraquat, a herbicide which is actively taken up by lung tissue [16], caused an increase in bound CP. The biogenic amine 5-hydroxytryptamine caused minimal inhibition of CP binding and mephentermine and amphetamine, drugs which are structurally similar to CP, were considerably less potent than many of the structurally dissimilar compounds used. To place the magnitude of these effects in context, it should be noted that the addition of unlabelled CP at the same concentration as the other inhibitors (5×10^{-4} M), caused a 38 per cent decrease in binding by competing directly with [^{14}C]-CP for the same binding sites.

The effect of DMI on CP binding in the microsomal fraction is shown in Fig. 2. Only the higher DMI level (5×10^{-4} M) significantly ($P < 0.01$) decreased the binding of CP at all concentrations. At this concentration of DMI, there was a significant ($P < 0.05$) increase from 19.4 ± 3.4 to 28.6 ± 2.1 per cent CP bound over the range of 0.14 μM to 4.98 μM C_t, a phenomenon which cannot be explained by classical competitive binding theory. In the 15,000 *g* fraction (Fig. 3), DMI (4×10^{-4} and 8.25×10^{-4} M) significantly ($P < 0.01$) decreased the binding of CP with greater inhibition at the higher concentration. As in Fig. 1, saturation was seen above a C_t level of 100 μM.

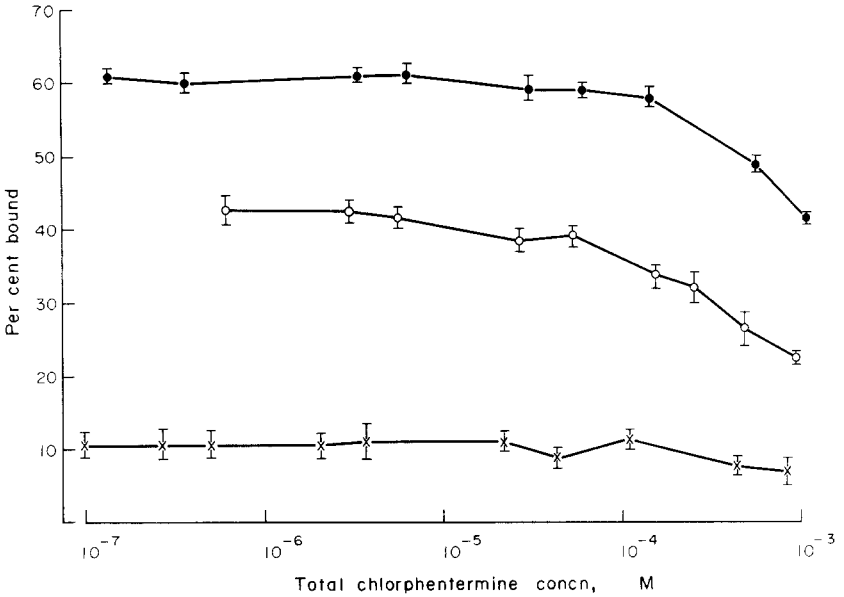

Fig. 1. Binding of chlorphentermine to the three rat lung subcellular fractions plotted as percent drug bound versus the total concentration of chlorphentermine on the protein side of the dialysis cells. Each point represents the mean ± S.E.M. of 4 experiments. Key: ● = microsomal fraction, ○ = 15,000 *g* fraction, X = cytosol fraction.

Non-linear regression analysis of the CP binding data from both fractions showed only one class of binding sites with definite evidence for a non-specific partitioning process. In the 15,000 *g* fraction, CP had a binding capacity of 108 ± 8 nmoles · mg^{-1} protein and an affinity constant of (2.01 ± 0.16) × 10^3 M^{-1} while in the microsomal fraction it was 340 ± 17 nmoles · mg^{-1} protein and (2.12 ± 0.11) × 10^3 M^{-1} respectively (Table 2). These values are comparable to those reported for imipramine and chlorpromazine in rat lung microsomes [17]. CP affinity constants estimated in the presence of increasing concentrations of competitor (DMI) according to equation 2 are apparent only and would be expected to decrease regularly. The presence of DMI (4×10^{-4} M) in the 15,000 *g* fraction caused a significant decrease ($P < 0.01$) in the apparent K_a of CP from (2.01 ± 0.16) × 10^3 M^{-1} to (0.57 ± 0.16) × 10^3 M^{-1}. However, when the concentration of DMI was increased to 8.25×10^{-4} M, no further decrease in K_a was seen. DMI also affected the $K_{0/w}$ of CP particularly at the higher concentration where it decreased from 0.077 ± 0.005 in the absence of DMI to 0.018 ± 0.002 ($P < 0.01$) for 8.25×10^{-4} M DMI. None of the DMI induced changes in binding capacity (n) shown in Table 2 were significant.

Table 1. Inhibition of chlorphentermine binding by various compounds in rat lung 15,000 *g* subcellular fraction

Inhibitor	Per cent inhibition	
Desmethylimipramine	67.4	(61.2,73.6)
Amitriptyline	64.5	(63.4,65.6)
Quinidine	59.3	(48.3,70.2)
Chlorcyclizine	58.2	(57.4,59.0)
Imipramine	54.6	(48.6,60.6)
Pronethalol	44.2	(37.6,50.7)
Propranolol	43.2	(33.9,52.4)
Chlorphentermine	37.7	(28.6,46.7)
d-Propoxyphene	33.3	(31.7,34.9)
Chloroquine	32.1	(27.6,36.6)
Methadone	25.4	(20.8,30.0)
Mepyramine	22.6	(20.6,24.6)
Diphenhydramine	18.8	(18.6,19.1)
Lignocaine	16.5	(12.3,20.7)
Mephentermine	14.1	(6.5,21.6)
5-Hydroxytryptamine	12.0	(8.5,15.5)
d-Amphetamine	9.0	(6.7,11.2)
Naloxone	4.1	(3.8,4.4)
Salicylic acid	2.8	(−5.0,10.5)
Paracetamol	−3.1	(−7.6,1.5)
Pentobarbitone	−4.6	(−10.7,1.6)
Caffeine	−6.6	(−8.6,−4.6)
Paraquat	−18.0	(−19.1,−16.9)

Inhibitor (5×10^{-4} M) was added to both sides of the dialysis cell while [^{14}C]chlorphentermine (5×10^{-7} M) was added to the protein-free side. Results are expressed as the mean of duplicates with the range given in parentheses.

Since DMI had no significant effect on n in the 15,000 *g* fraction, K_d was estimated with equation 3 using the experimental CP binding capacity found in the absence of DMI (n = 108 nmoles · mg^{-1} protein). Table 2 shows that (1) the affinity constants of CP estimated according to equation 3 are the same regardless of DMI concentration ((2.01 ± 0.16, 1.94 ± 0.08, 2.01 ± 0.13) × 10^3 M^{-1}), a result supporting applicability of the model, and (2) the affinity constant for DMI (K_d) significantly increased from (2.28 ± 0.21) × 10^3 M^{-1} to (4.76 ± 0.27) × 10^3 M^{-1} ($P < 0.01$) with increasing DMI concentration. The magnitude of K_d is comparable to reported affinity constants for DMI in rat liver microsomes [17].

Hill plots were constructed to assess the possibility of a co-operative interaction between CP and DMI binding in the 15,000 *g* fraction. For zero and 4×10^{-4} M DMI, the Hill coefficients for CP binding

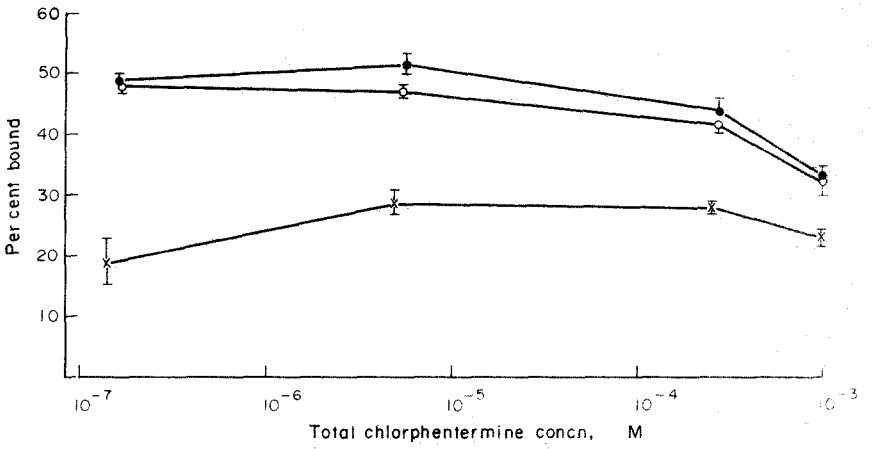

Fig. 2. Effect of desmethylimipramine on the binding of chlorphentermine to rat lung microsomal fraction plotted as per cent drug bound versus the total concentration of chlorphentermine on the protein side of the dialysis cells. Each point represents the mean ± S.E.M. of 4 experiments. Key: ● = no DMI, ○ = 3×10^{-5} M DMI, X = 5×10^{-4} M DMI.

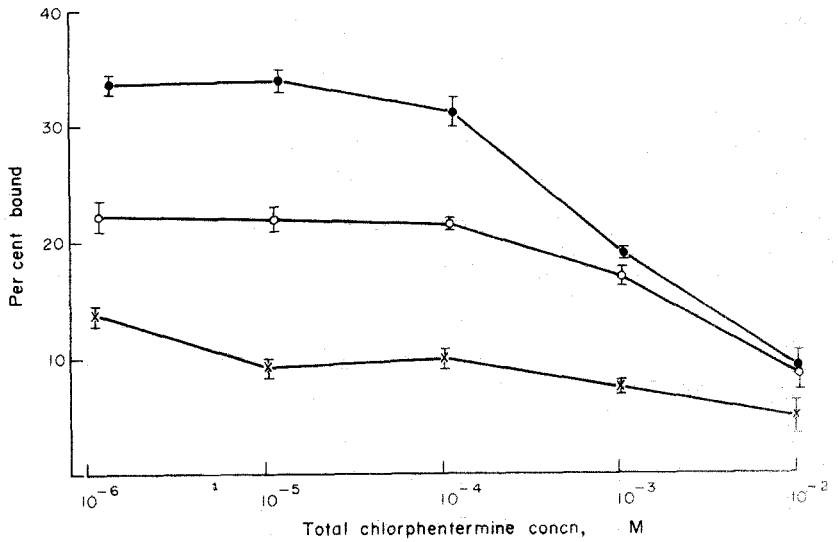

Fig. 3. Effect of desmethylimipramine on the binding of chlorphentermine to rat lung 15,000 *g* fraction plotted as per cent drug bound versus the total concentration of chlorphentermine on the protein side of the dialysis cells. Each point represents the mean ± S.E.M. of 4 experiments. Key: ● = no DMI, ○ = 4×10^{-4} M DMI, X = 8.25×10^{-4} M DMI.

Table 2. Binding parameters of chlorphentermine (CP) and desmethylimipramine (DMI) in rat lung subcellular fractions

Subcellular fraction	DMI concentration (M)	CP binding capacity (nmoles · mg^{-1} protein, equation 2)	CP affinity constant ($\times 10^3$ M^{-1}, equation 2)	CP oil/water partition coefficient (equation 2)	CP affinity constant ($\times 10^3$ M^{-1}, equation 3)	DMI affinity constant ($\times 10^3$ M^{-1}, equation 3)
Microsomal	—	340 ± 17	2.12 ± 0.11	0.060 ± 0.001	2.12 ± 0.11	—
15,000 *g*	—	108 ± 8	2.01 ± 0.16	0.077 ± 0.005	2.01 ± 0.16	—
	4×10^{-4}	208 ± 51	0.57 ± 0.12	0.049 ± 0.017	1.94 ± 0.08	2.28 ± 0.21
	8.25×10^{-4}	71 ± 22	0.75 ± 0.27	0.018 ± 0.002	2.01 ± 0.13	4.76 ± 0.27

Binding capacities, apparent affinity constants and oil/water partition coefficients of CP were determined by non-linear regression of bound CP (C_b) on free CP (C_f), assuming the model derived from the law of mass action: $C_b = (nPK_aC_f)/(1 + K_aC_f) + K_{0/w}C_f$ — equation 2. The affinity constants of DMI and CP were determined using the competitive binding equation: $C_b = (nPK_aC_f)/(1 + K_aC_f + K_dC_d) + K_{0/w}C_f$ — equation 3, where K_d and C_d are the affinity constant and free drug concentration respectively of DMI. Data are presented as mean ± S.E.M.

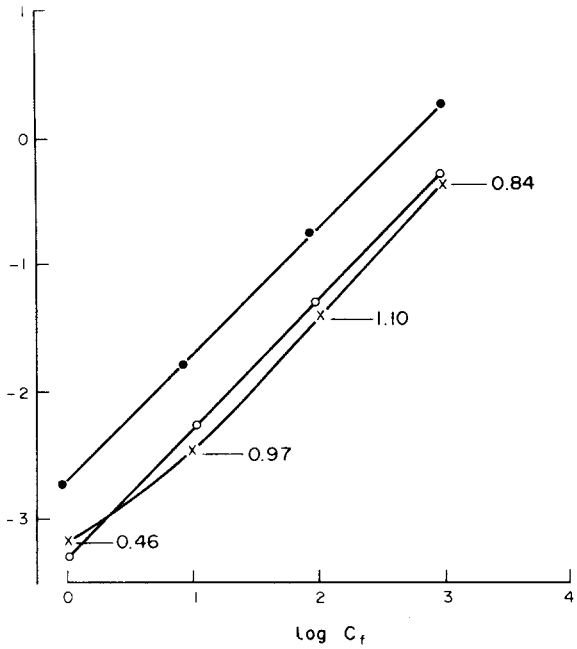

Fig. 4. Hill plot of chlorphentermine binding to 15,000 *g* rat lung fraction in the presence and absence of desmethylimipramine. Plots were analysed by stepwise polynomial regression to determine non-linearity. For [DMI] = 0 and 4×10^{-4} M, the slope was not significantly ($P > 0.05$) different from 1. For [DMI] = 8.25×10^{-4} M the plot was best represented by a third order polynomial which was used to give the slopes indicated. Each plot consists of 16 experimental observations and points shown are means of four observations. C_f = free CP concentration, C_b = bound concentration, nP = binding capacity. Key: ● = no DMI, ○ = 4×10^{-4}M DMI, × = 8.25×10^{-4}M DMI.

were not significantly different from unity over the entire binding range, thus suggesting that CP binding sites are homogeneous and independent and that DMI inhibits CP binding by a simple direct competitive process. However, at 8.25×10^{-4} M DMI, the Hill coefficient was significantly less than one at the lower concentrations of CP, suggesting a negatively co-operative interaction (Fig. 4).

DISCUSSION

The results of the present study show that rat lung is able to bind large amounts of CP in both the 15,000 *g* and microsomal fractions but very little in the cytosol. A similar distribution of binding sites between subcellular fractions has been observed for propranolol [18]. Saturability was seen at concentrations above 100 μM and the binding of CP was inhibited by a diverse group of compounds indicating little steric specificity in the process. The extent of CP binding was comparable to that reported for imipramine and chlorpromazine in rat lung microsomal fractions [17] but whereas the former was described by one class of binding sites and a partitioning process, Bickel and Steele [17] concluded that the latter had two classes of sites. Their estimated affinity constants were 3.9×10^4 M^{-1} and 2×10^3 M^{-1} for imipramine and 2.2×10^4 M^{-1} and 2.2×10^3 M^{-1} for chlorpromazine. They did not consider parameter uncertainties in their analyses and consequently it is difficult to judge the adequacy of their binding model. In the absence of DMI, independent binding of CP to the microsomal and 15,000 *g* fractions occurred throughout the total concentration range studied (C_t of 10^{-6}–10^{-2} M).

CP binding in different subcellular fractions correlates well with the distribution of phospholipids. Hook *et al.* [19] reported that rat lung microsomes contain the highest concentration of phospholipids (0.35 mg/mg protein), the 600 *g* and mitochondrial fractions (comparable to the 15,000 *g* fraction used in the present study) contain less (0.17 mg/mg protein) and the cytosol very little (0.05 mg/mg protein). Nuclear magnetic resonance studies have revealed that CP interacts with phosphatidylcholine and that the complex is formed primarily through hydrophobic binding of the aromatic ring structure, although the cationic amine group is essential for the drug–phospholipid interaction [8]. It has been suggested that the cationic group provides the initial attraction between the two molecules after which the shorter-reaching hydrophobic forces become effective and predominate [8]. Amitriptyline, chloroquine and chlorcyclizine form a similar complex with phosphatidylcholine [8] and DMI and imipramine have been found to bind to bile micelles containing this phospholipid [20]. It is therefore likely that phospholipids play an important role in the binding of basic amines to lung tissue and that the relatively simple interaction between the two molecules accounts for the non-specificity of binding observed in this study.

All of the compounds which caused any appreciable inhibition of CP binding are basic amines, most have previously been found to accumulate in lung tissue [2], and many, such as DMI and imipramine [4], diphenhydramine and imipramine [5], DMI and propranolol [6], chlorcyclizine and amphetamine [5], methadone and imipramine [5] have been shown to interact in the isolated perfused lung resulting in decreased accumulation of one of the drugs. As in the present study, high concentrations of inhibitor were usually necessary to demonstrate inhibition.

Of the drugs tested, DMI was found to be the most potent inhibitor of CP binding in the 15,000 *g* fraction and the nature of the interaction between the two compounds was dependent on their relative concentrations. At 4×10^{-4} M DMI, the interaction appeared competitive at all concentrations of CP as indicated by the unit slope Hill plot as well as the absence of any change in the affinity constant of CP. However, the estimated affinity constant of DMI ($(2.28 \pm 0.21) \times 10^3$ M^{-1}) was surprisingly similar to that of CP ($(2.01 \pm 0.16) \times 10^3$ M^{-1}) since, as shown in Table 1, it is more potent as an inhibitor of CP binding than CP itself (67 per cent compared to 38 per cent inhibition). This raises the question of whether or not DMI interacts with CP binding by direct competition. When the DMI concentration was increased to 8.25×10^{-4} M, definite evidence for a non-competitive interaction was obtained. The apparent affinity constant of CP, estimated using equation 2, did not continue to decrease with increasing levels of DMI as would be expected with simple competition. The affinity constant of DMI, rather than being a constant independent of concentration, increased dramatically as DMI concentration was increased. The low Hill coefficient (0.47) at the lower CP concentrations indicates a negatively co-operative

interaction (antagonism between DMI and CP). Furthermore, estimation of the binding parameters for CP according to equation 2, was made with the assumption of reversible binding to one class of independent sites common to both CP and DMI. The relative uncertainty in n and the apparent K_a of CP was found to increase from 7 and 8 per cent in the absence of DMI to 25 and 20 per cent at a DMI concentration of 4×10^{-4} M and to 30 and 36 per cent respectively at a DMI concentration of 8.25×10^{-4} M. Since the accuracy in assaying CP was similar in all situations, the increased uncertainty in binding parameters with increasing DMI concentration can be taken as evidence of progressive inadequacy of the model. Some increase in parameter uncertainty is predictable as Schary *et al.* [15] have shown that Scatchard plots for a one-class model in the presence of increasing concentrations of a competitor become progressively non-linear. However, in the present study, the proportion of the increase attributable to this cause was negligible.

The decreased binding of CP in the lower concentration range and the increase in affinity constant for DMI is contrary to what would be expected from the reciprocity relationship for co-operative interactions derived by Weber [21]. This relationship predicts that the change in binding of one drug by a second will be matched in direction and extent by its effect on the second drug. If the mechanism of interaction between CP and DMI is co-operative rather than directly competitive, then mere inspection of relative affinity constants does not suffice for prediction of displacement which will depend on coupling between binding sites; only if this is strong, will there by significant displacement. The other major consequence of a co-operative rather than a competitive interaction is that the degree of displacement is saturable, whereafter further increase in the inhibitor concentration produces no further displacement. Co-operative processes are more commonly mediated by conformational transitions in the binding moiety or less commonly by alterations in the structure of bound water.

In the 15,000 g fraction, CP was approximately 44% bound at the lower levels and in the microsomal fraction it was approximately 61% bound (Fig. 1). On the basis that there was three times as much protein per gram of tissue in the former as in the latter, it would appear that *in vivo* the 15,000 g fraction would be more important. The relationship between per cent bound and protein concentration at low CP concentrations is not linear. Knowing that CP has one class of binding sites in each fraction it can be shown that the limiting fraction bound (β) at low CP concentration is equal to $nK_a/(nK_a + 1)$. Trebling protein concentration then amounts to a theoretical change in β to 70 per cent, only slightly greater than the 61 per cent observed for the microsomal fraction. In view of the closeness of these two figures it is likely that the microsomal fraction will also be important in the total disposition of CP in rat lung.

The partitioning component involved in the interaction of CP with the 15,000 g fraction is of major importance at higher drug levels where it can account for up to 70 per cent of CP bound. It would seem that the partitioning mechanism in the microsomal and 15,000 g fractions is common, given the similar $K_{o/w}$ constants obtained. The observed effect of DMI on the partitioning of CP could be understood by changes in the interfacial tension between phases as has been suggested by Seydel and Wassermann [22] for the effect of cholesterol on the binding of CP to phosphatidylcholine micelles.

In view of the non-competitive interaction between CP and DMI found in the present study and the general statement by Weber [21] that independent binding may prove to be the exception rather than the rule, it is likely that many more co-operative drug interactions in lung uptake will be found, and that these interactions will have as their common basis, binding-induced conformational transitions.

REFERENCES

1. A. F. Junod, in *Lung Metabolism* (Eds A. F. Junod and R. de Haller), p. 387. Academic Press, New York (1975).
2. E. A. B. Brown, *Drug Metab. Rev.* **3**, 33 (1974).
3. H. Remmer, in *Lung Metabolism* (Eds A. F. Junod and R. de Haller), p. 133. Academic Press, New York (1975).
4. A. F. Junod, *J. Pharmac. exp. Ther.* **183**, 182 (1972).
5. M. W. Anderson, T. C. Orton, R. D. Pickett and T. E. Eling, *J. Pharmac. exp. Ther.* **189**, 456 (1974).
6. C. T. Dollery and A. F. Junod, *Br. J. Pharmac.* **57**, 67 (1976).
7. A. F. Junod, in *Lung Metabolism* (Eds A. F. Junod and R. de Haller), p. 219. Academic Press, New York (1975).
8. H. Lüllmann, R. Lüllmann-Rauch and O. Wassermann, *Crit. Rev. Toxicol.* **4**, 185 (1975).
9. J. Gloster, D. Heath, P. Hasleton and P. Harris, *Thorax* **31**, 558 (1976).
10. D. Karabetnik and G. Zbinden, *Archs Toxicol.* **35**, 163 (1976).
11. T. Davila and D. Davila, *Archs int. Pharmacodyn. Thér.* **215**, 336 (1975).
12. S.-J. Liu and R. I. H. Wang, *J. Pharmac. exp. Ther.* **195**, 94 (1975).
13. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
14. B. W. Madsen and J. S. Robertson, *J. Pharm. Pharmac.* **26**, 807 (1974).
15. W. L. Schary, L. J. Aarons and M. Rowland, *Biochem. Pharmac.* **27**, 139 (1978).
16. M. S. Rose, L. L. Smith and I. Wyatt, *Nature, Lond.* **252**, 314 (1974).
17. M. H. Bickel and J. W. Steele, *Chem.-Biol. Interact.* **8**, 151 (1974).
18. D. W. Schneck, J. F. Pritchard and A. H. Hayes, Jr., *J. Pharmac. exp. Ther.* **203**, 621 (1977).
19. G. E. R. Hook, J. R. Bend, D. Hoel, J. R. Fouts and T. E. Gram, *J. Pharmac. exp. Ther.* **182**, 474 (1972).
20. M. H. Bickel and R. Minder, *Biochem. Pharmac.* **19**, 2437 (1970).
21. G. Weber, *Adv. Protein Chem.* **29**, 1 (1975).
22. J. K. Seydel and O. Wassermann, *Biochem. Pharmac.* **25**, 2357 (1976).