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Correlation of physicochemical properties with absorption and metabolism of some tricyclic drugs

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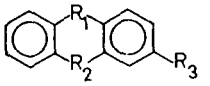
Octanol and dodecane partition coefficients, surface activity and adsorbability to activated charcoal were determined for six tricyclic psychotropic drugs with *N*-dimethylalkyl side chains. Surface activity correlated well with the partition coefficients, and all drugs obeyed the Langmuir adsorption isotherm. A correlation between the reciprocal of the death time of gold fish exposed to drugs and partition coefficients was observed. The extent to which the drugs were *N*-demethylated as measured by formaldehyde formed in rat liver homogenate incubations correlated with their adsorbability to activated charcoal but not with their ability to inhibit aniline-*p*-hydroxylase, nor was there a linear correspondence between *N*-demethylation and drug lipophilicity as indicated by partition coefficients or surface activity.

Studies which relate physicochemical properties of series of similar drugs with biological activity contribute to rationalize drug design. They lead to the identification of structural features in drug molecules which are essential for activity and thus enable tentative predictions of biological properties of new derivatives in the series. Phenothiazine neuroleptics comprise a group of drugs which has been the subject of several reports in which physicochemical properties were compared with pharmacological or therapeutic activity. A rank order correlation between dodecane partition coefficients, surface activity, and toxicity in gold fish was reported for various 2-substituted chlorpromazine derivatives (Nightingale, Tse & Stupak, 1972). Nogami, Nagai & Nambu (1970) showed that the adsorbability of seven neuroleptics could be correlated with their platelet 5-hydroxytryptamine releasing activity. Phenothiazines were found to lower surface tension in proportion to their clinical potency (Seeman & Bialy, 1963).

In contrast to the numerous investigations dealing with phenothiazine neuroleptics only few studies have been published on tricyclic antidepressants relating their structures to biological activity. For four tricyclic antidepressants Nambu, Sakurai & Nagai (1975) found a correspondence between their adsorbability to carbon black and both their inhibitory effect on the histamine contraction of guinea-pig ileum and their ability to inhibit the adrenaline contraction of guinea-pig vas deferens. However for both the phenothiazines and tricyclic antidepressants, no attempt has been made to quantitatively correlate physicochemical data with the extent to which they are metabolized. Hewick & Beckett (1971) observed that *N*-demethylation of *N*-dimethyl alkyl substituted phenothiazine drugs increased with increase in length of the alkyl side chain and decreased by introducing electronegative substituents into the phenothiazine ring.

For the present investigation six structurally related but pharmacologically dissimilar tricyclic psychotropic drugs, three phenothiazine derivatives (chlorpromazine, promazine, promethazine) and three tricyclic antidepressants (imipramine, doxepine, amitriptyline) with *N*-dimethyl alkyl side chain have been chosen (Table 1). All drugs are metabolically *N*-demethylated to pharmacologically active *N*-monomethyl derivatives (Gram, Reisby & others, 1976).

Table 1. *Structure of tricyclic psychotropic drugs, used in the study.*



Compound	R_1	R_2	R_3
Imipramine	$-[CH_2]_3-$	$N[CH_2]_3NMe_2$	H
Amitriptyline	$-[CH_2]_3-$	$C=CH[CH_2]_2NMe_2$	H
Doxepine	$-CH_2O-$	$N[CH_2]_3NMe_2$	H
Promazine	$-S-$	$N[CH_2]_3NMe_2$	H
Promethazine	$-S-$	$N[CH_2]_2CHMeNMe_2$	H
Chlorpromazine	$-S-$	$N[CH_2]_3NMe_2$	Cl

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The aim of the investigation was threefold:— (1) To elucidate the influence of physicochemical properties on the rate of metabolic *N*-demethylation *in vitro*; (2) to establish whether the relation between hydrophobicity and absorption as measured by toxicity in gold fish, which was observed for substituted chlorpromazine derivatives (Nightingale & others, 1972), is valid for other tricyclic psychotropic drugs; (3) to re-investigate the unexpected finding that unlike barbiturates (Nogami, Nagai & Uchida, 1969) phenothiazine drugs and tricyclic antidepressants did not show a clear relation between different hydrophobicity indicating parameters (Nambu & others, 1975).

MATERIALS AND METHODS

The following tricyclic drugs, kindly supplied by the manufacturers, were used: imipramine HCl (Ciba-Geigy), doxepine HCl (Pfizer Ltd.), chlorpromazine HCl and promethazine HCl (May and Baker Ltd), and promazine HCl (Wyeth Laboratories).

(i) Partition coefficient determination

The apparent partition coefficient of each drug was measured by shaking 50 ml of a 1·0 mM drug solution made up in Sorensen citrate buffer pH 6·0 in the presence of 2 ml of octanol or 5 ml dodecane at 25° for 12 h protected from light. Buffer saturated with the organic phase and buffer saturated organic solvent were used. After equilibration the aqueous phase was analysed for residual drug content by ultraviolet spectrophotometry by determining spectral absorption between 210 and 300 nm.

(ii) Adsorbability constant measurement

Adsorption was determined by shaking 100 ml of buffered drug solution 0·625 to 1·25 mM, in the presence of 50 mg of activated charcoal (BDH Ltd.) for 48 h at 25° under light exclusion. After equilibration the solutions were filtered under vacuum through GF/C filters (Whatman) and assayed as above.

(iii) Surface tension measurement

The surface tension of buffered solutions of the drugs was measured at 25° using a De Nouy surface tensiometer fitted with a 12·5 mm diameter platinum ring. The drug concentration chosen was 1 mM, which is below the critical micelle concentration for all tricyclic drugs used (Gescher & Li Wan Po, unpublished). Lower concentrations as used in a previous study (Seeman & Bialy, 1963) did not produce a measurable surface tension depression for three of the six drugs.

(iv) Gold fish death time

This method was used as it is a widely accepted model for drug absorption (Florence 1970; Lovering, Mainville & Rowe, 1976; Marriott & Kellaway, 1976). The same procedure as described by Levy & Gucinski (1964) was used. The mean death time of five gold fish, 3–5 g, was determined. Each fish was placed in 100 ml of a 1 mM solution of the drug and a temperature of 22° was maintained throughout the experiment. Death time was chosen as an end point instead of overturn time (Gibaldi & Nightingale, 1968) because it gave more reproducible values. Drug concentration was 1 mM thus avoiding complications arising from adsorption onto glass (Nightingale & others, 1972) and eliminating effects of micelle formation on drug absorption (Florence, 1970).

(v) *Tricyclic drug* N-*demethylase activity and inhibition of aniline* p-*hydroxylase*

Male Wistar rats, 100–200 g, were killed after pretreatment with phenobarbitone (70 mg kg^{-1} day^{-1}, i.p.), for 3 days. The livers were homogenized in 4 parts ice cold 0·1 M tris buffer pH 7·4 and centrifuged at 9000 *g* for 25 min. The incubation medium (3 ml) contained 1·2 μmol NADP, 15 μmol $MgCl_2$, homogenate equivalent to 200 mg liver, and 3 μmol substrate, either tricyclic drug or aniline HCl. With the incubation of mixtures of two tricyclic drugs the concentration of each drug was 0·5 mM instead of 1 mM. The mixture was incubated for 30 min at 37° under air and the reaction was terminated by adding 0·5 ml 30% w/v trichloroacetic acid to the incubation medium. *N*-Demethylation of the tricyclic drugs was estimated by measuring released formaldehyde (Nash, 1953). Demethylase dependent formaldehyde production was linear with time and proportional to microsomal protein concentration as determined by the method of Lowry, Rosebrough & others (1951) after preparation of microsomes by centrifugation at 100 000 *g* for 1 h. The extent of *N*-demethylation is expressed as % of formaldehyde formed from 1 mM imipramine in 30 min at 37°. The rate of imipramine *N*-demethylation was taken as 100% in each individual experiment to account for individual differences between animals. In 10 experiments 283 ± 64 nmol of HCHO were formed from 1 mM imipramine by 0·2 g liver in 30 min.

For the inhibition experiments aniline HCl was used as substrate in the presence of 50 μM to 1 mM tricyclic drug. The amount of *p*-hydroxyaniline formed was measured colorimetrically according to Archakov, Karuzina & others (1974). The ID_{50} values in Table 2 refer to the concentration of tri-

Table 2. *Physiochemical parameters, gold fish death time, rate of* N-*demethylation, and ID50 values for inhibition of aniline* p-*hydroxylase of tricyclic pschotropic drugs.* Number of experiments in brackets. Experimental details under methods.

Compound	Partition coefficient K^{app}_{dod}	Partition coefficient K^{app}_{oct}	Decrease in surface tension (dynes cm^{-1}) (mNm^{-1})	Langmuir adsorption coefficients (mol g^{-1} 10^3)	Gold fish death time (min)	*N*-Demethylation rate. % HCHO formed from imipramine under identical conditions	Inhibition of aniline *p*-hydroxylase ID50 (μM)
Imipramine HCl	3·51	16·10	2·0	1·467	22 ± 2	100 (10)	236
Doxepine HCl	2·05	11·16	1·1	1·305	27 ± 3	135 ± 88 (10)	102
Amitriptyline HCl	11·19	44·20	4·2	1·534	8 ± 1	94 ± 13 (7)	172
Promazine HCl	2·48	16·80	1·8	1·689	17 ± 2	62 ± 8 (8)	392
Promethazine HCl	7·01	38·25	3·3	1·656	20 ± 2	60 ± 14 (7)	—
Chlorpromazine HCl	23·83	102·35	9·0	1·774	6 ± 1	28 ± 7 (9)	371

cyclic drug which inhibits *p*-hydroxylase by 50%. The values were derived from log inhibitory dose—response curves which were calculated from four or five concentrations of inhibitor drug. Values for each concentration point were obtained from 6 animals. In all cases the regression analysis gave correlation coefficients r >0·93.

RESULTS

Table 2 presents the physicochemical (columns 1 to 4) and biological data (columns 5, 6 and 7) obtained for the six tricyclic psychotropic drugs. The partition coefficients (column 2) obtained with dodecane as the lipophilic solvent were significantly lower than those obtained with octanol indicating that the tricyclic drugs are more soluble in polar solvents than in non-polar solvents. It is also known that partitioning of the protonated form of the drugs is possible in the octanol: buffer system (Murthy & Zografi, 1970). The extent of the correlation between the dodecane and the octanol partition coefficients is shown in Fig. 1. Such a relation is not unexpected considering the work of Collander (1951) and Seiler (1974) which showed that the log partition coefficients in different solvent systems are linearly related. Column 4 of Table 2 presents adsorbability constants of the six drugs, all of which obeyed the Langmuir adsorption isotherm. Fig. 2 shows the ratio of the equilibrium concentration and the amount adsorbed g^{-1} of charcoal (c/x), plotted against the equilibrium concentration C for imipramine and chlorpromazine; the other four drugs gave similar plots. The decrease in surface tension of buffer solution upon addition of each drug is given in column 3 of Table 2. The values correlate well with both the octanol:

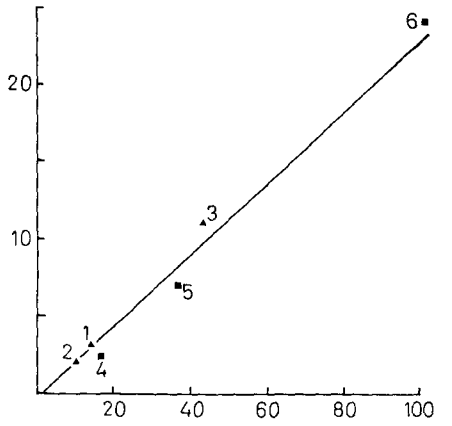

FIG. 1. Correlation between dodecane (ordinate) and octanol (abscissa) partition coefficients. 1. imipramine HCl; 2. doxepine HCl; 3. amitriptyline HCl; 4. promazine HCl; 5. promethazine HCl; 6. chlorpromazine HCl. Linear regression coefficient (γ) = 0·993.

buffer and the dodecane: buffer partition coefficients (Fig. 3).

Of the biological data in Table 2 the reciprocal of the death time of gold fish exposed to the drugs (column 5) did not correlate with the Langmuir adsorption constants. However it showed a correlation with the dodecane partition coefficients (Fig. 4). This finding is in accordance with previously reported evidence for a semi quantitative relation between partition coefficients and gold fish toxicity of phenothiazines (Nightingale & others, 1972).

The extent to which the six drugs were *N*-demethylated to active metabolites (Table 2, column 6) was correlated with the adsorbability on to charcoal (Fig. 5). To establish whether this finding reflects the

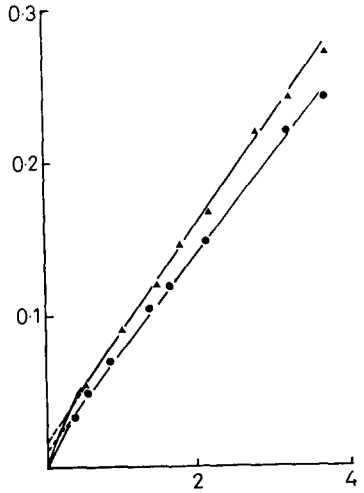

FIG. 2. Langmuir adsorption plots for imipramine (▲) and promazine (●) hydrochlorides. Ordinate: Equilibrium concentration/amount adsorbed (g litre^{-1}). Abscissa: Equilibrium concentration (CE) (mol litre^{-1} $\times 10^4$).

ability of the drugs to inhibit their own metabolism, imipramine was incubated with doxepine or chlorpromazine. Of the six drugs studied the former showed the highest and the latter the lowest rate of *N*-demethylation (Table 2, column 6). The amount of formaldehyde found in the incubation mixture of imipramine and chlorpromazine (each 0·5 mM) (49 ± 6%, $P < 0{\cdot}05$) was less than half of that formed by imipramine alone (1 mM) (100%) while in the mixture of imipramine and doxepine (each 0·5 mM) more was formed (108 ± 11%). At the substrate concentration used there is no linear relation between substrate concentration and *N*-demethylation rate of tricyclic psychotropic drugs. Therefore the amount of formaldehyde formed in mixtures of these drugs cannot be expected to be the sum of that obtained in metabolic incubations of the individual drugs. Nevertheless, the results allow the conclusion that chlorpromazine inhibits imipramine *N*-demethylation much more than doxepine. So one can assume that chlorpromazine inhibits its own metabolism more than doxepine inhibits its metabolism. Thus the correlation between adsorbability and *N*-demethylation seems to be related to the differences in substrate

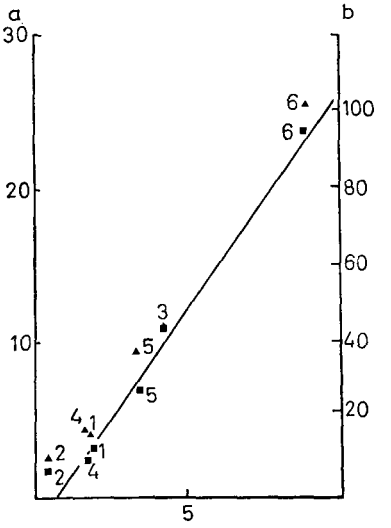

FIG. 3. Relation between partition coefficient and decrease in surface tension. Ordinates a: Dodecane (■); b: Octanol (▲) apparent partition coefficients. Abscissa: Decrease in surface tension (dynes cm^{-1}). Linear regression coefficient for dodecane Kapp, $\gamma = 0{\cdot}996$.

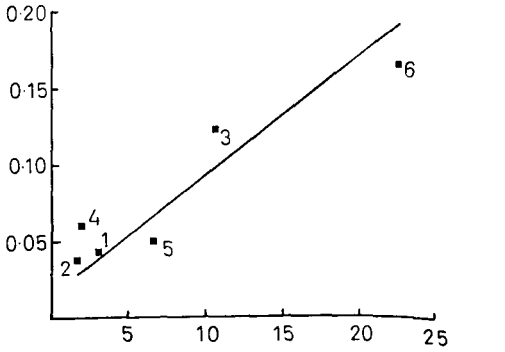

FIG. 4. Relation between dodecane partition coefficients (abscissa) and the reciprocal of death time (min) of gold fish (ordinate). $\gamma = 0{\cdot}943$.

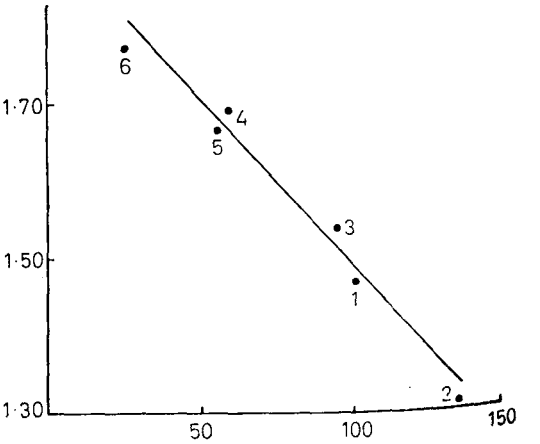

FIG. 5. Correlation between adsorbability constants and rate of *N*-demethylation. Ordinate: Langmuir adsorption constants (mol g^{-1} $\times$ 10^3). Abscissa: *N*-demethylation relative to imipramine (100%). $\gamma = 0{\cdot}988$.

self-inhibitory activity. One would therefore expect that the tricyclic drugs would also display similar differences in inhibition of other microsomal hydroxylation reactions. This was however shown not to be the case for the inhibition of aniline *p*-hydroxylation (Table 2, column 7) determined in rat liver homogenates. Furthermore the ability of five of the six drugs and other tricyclic antidepressants (Gescher & Li Wan Po, unpublished) to inhibit aniline *p*-hydroxylase did not correlate with any of the sets of physicochemical data.

DISCUSSION

Most studies on the influence of physicochemical data on biological activity of tricyclic psychotropic drugs have been made by workers who measured physicochemical parameters and compared them with pharmacological data published elsewhere. The present report is one of the few studies in which both kinds of data have been collected in the same laboratory. The physicochemical properties measured were surface activity, adsorbability to charcoal, and partition coefficients. Of these data surface activity and partition coefficients were correlated with each other but not with the adsorbability constants. Evidently in this class of compounds there is no linear relation between all the different hydrophobicity indicating parameters, which is consistent with previous results (Nambu & others, 1975). It can be argued that of these parameters adsorption is not a suitable index of hydrophobicity as adsorption at a solid/liquid interface is related to specific sites on the drug molecule rather than reflecting the lipophilicity of the whole molecule. On the other hand it has been shown for a series of substituted phenols that there is a linear relation between the Langmuir constant using carbon black as the adsorbent and log partition coefficients (Umeyama, Nagai & Nogami, 1971). Partition coefficients but not adsorbability constants corresponded with the reciprocal of the death time of gold fish exposed to the drugs. Dodecane partition coefficients have been shown to be more suitable for predicting differences in absorption between three chlorpromazine analogues (Nightingale & others, 1972) than values obtained with octanol as organic solvent. Our results indicate that with the drugs used in this study both partition coefficients gave equally good correlations. The correlation between partition coefficients and gold fish death time suggests that lipophilicity is important for drug absorption in the gold fish model.

It should however be borne in mind that many other factors could also affect drug absorption. Partition coefficients are equilibrium values whereas the uptake of a drug and its subsequent distribution is essentially a dynamic process. Lipid solubility is also known to be an important factor determining the property of a drug to act as substrate of the microsomal drug oxidizing system (Testa & Jenner, 1976). However not all studies relating lipophilicity to affinity of drugs to the mixed function oxidases or to the rate at which they are metabolized by this system have yielded unequivocal results. Among the classes of drugs for which there were no clear correlations between partition coefficients and rate of metabolism are barbiturates (Jansson, Orrenius & Ernster, 1972) and puromycine analogues (Mazel, Kerza-Kiviatecki & Simanis, 1966).

The present investigation did not reveal a linear correspondence between lipophilicity and rate of metabolism *in vitro* for tricyclic psychotropic drugs. The extent to which the drugs were *N*-demethylated was however correlated with their adsorbability to activated charcoal. This physicochemical parameter may influence the interaction between tricyclic drugs and mixed function oxidases in two ways: it may affect the affinity of the drug for the enzyme or the ability to inhibit its own metabolism. It is conceivable that the part of the molecule most likely to be adsorbed non-specifically to the enzyme, before interaction at the active site, is the tricyclic ring structure. The more strongly adsorbed molecules probably undergo conformational changes involving the *N*-dimethyl substituted side chain less easily. However with the less strongly adsorbed drugs the *N*-dimethyl alkyl side chain may be more easily brought in contact with the active site on the enzyme surface thus favouring *N*-demethylation. Mixed function oxidases also catalyse the *p*-hydroxylation of aniline. We found no correlation between the ability of the tricyclic drugs to inhibit this reaction and their adsorbability. This finding is consistent with the results of Shand & Oates (1972) who could not find any structural features which influenced the inhibitory action of a similar range of tricyclic drugs on the *in vitro* metabolic hydroxylation of propranolol.

The free energy related logarithm of the absolute partition coefficients rather than the untransformed apparent values have been frequently correlated with biological activity (e.g. Leo, Hansch & Elkins, 1971; Tong & Lien, 1976). In this study however no attempt has been made to transform the apparent partition values due to the absence of precise pKa values for most of the tricyclic drugs investigated.

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