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A method to determine the ability of drugs to diffuse through the blood–brain barrier

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ABSTRACT **A method has been devised for predicting the ability of drugs to cross the blood–brain barrier. The criteria depend on the amphiphilic properties of a drug as reflected in its surface activity. The assessment was made with various drugs that either penetrate or do not penetrate the blood–brain barrier. The surface activity of these drugs was quantified by their Gibbs adsorption isotherms in terms of three parameters: (*i*) the onset of surface activity, (*ii*) the critical micelle concentration, and (*iii*) the surface area requirement of the drug at the air/water interface. A calibration diagram is proposed in which the critical micelle concentration is plotted against the concentration required for the onset of surface activity. Three different regions are easily distinguished in this diagram: a region of very hydrophobic drugs which fail to enter the central nervous system because they remain adsorbed to the membrane, a central area of less hydrophobic drugs which can cross the blood–brain barrier, and a region of relatively hydrophilic drugs which do not cross the blood–brain barrier unless applied at high concentrations. This diagram can be used to predict reliably the central nervous system permeability of an unknown compound from a simple measurement of its Gibbs adsorption isotherm.**

The tight endothelium of brain capillaries constitutes the permeability barrier for the passive diffusion of substances from the blood stream into the central nervous system (CNS) (1). To reach the brain, a molecule has first to be absorbed from the blood into the endothelial cell, where it is then released into the brain. A prerequisite for a substance to diffuse through the blood–brain barrier (BBB) is therefore a certain degree of lipid solubility.

The most common *in vivo* method for determining how a drug penetrates into the CNS is to apply a radioactive compound peripherally and measure its accumulation in the brain. This procedure is, however, not suitable for the screening of a large number of substances. A simpler, *in vitro* method is to measure the partition equilibrium of a drug between an aqueous phase and an immiscible organic phase (e.g., octanol) either by shake-flask methods (2) or by HPLC (3–6). Although none of the lipophilic phases used to date are entirely satisfactory in mimicking the biological membrane, the measured partition coefficients are usually interpreted in a straightforward manner. A weak partitioning into the organic phase means low lipophilicity and is assumed to correlate with a low CNS penetration power; a high affinity for the organic phase is translated into a good CNS availability. However, many exceptions have been observed in both the low and the high lipophilicity range (7, 8).

Here we propose an approach which takes advantage of the surface activity of the molecule of interest. Three properties of a drug molecule have been suggested to determine its ability to cross the BBB (9): (*i*) the number of lipophilic groups, (*ii*) the number of charged groups and their extent of ionization, and (*iii*) the molecular size. Interestingly, the same three parameters also determine the surface activity and their combined action can be evaluated in a single experiment by measuring the Gibbs adsorption isotherm.

Here the chromatographically determined lipophilicity constants are compared with measurements of the surface activity for a large number of structurally different compounds known to cross or not to cross the BBB. The predictive value of the surface activity measurements as a function of concentration is distinctly higher than that of the conventional lipophilicity measurements.

MATERIALS AND METHODS

Materials. Twenty-eight drugs were selected and classified according to CNS availability (Table 1). Compounds known to penetrate easily into the CNS were denoted CNS^+; those which did not penetrate or penetrated only weakly were denoted CNS^-. Only those drugs were considered for which the CNS permeation tendency had already been established on the basis of either broad clinical experience or unambiguous experimental results.

Most drugs were provided by the Medicinal Chemistry Laboratories of E. Merck. The rest were from commercial sources.

Estimation of Lipophilicity by a Polycratic Reversed-Phase HPLC Method. All experiments were performed on a Merck–Hitachi HPLC system at 37°C and pH 7.2. The lipophilicity constant, log k'_w, which corresponds to the logarithm of the capacity ratio measured in a totally aqueous eluent (see Eq. 2), was determined by polycratic reversed-phase HPLC on a C_{18}-silica column (36–38). Retention times, t, of the compounds were measured in eluents with varying methanol/buffer ratios. The volume fraction of methanol, Φ, of the eluents was incremented by 5% (in general, $20\% < \Phi < 80\%$). Capacity ratios were calculated as

$$k'_\Phi = \frac{t_\Phi - t_0}{t_0}. \qquad [1]$$

Finally, log k'_w was obtained by linear extrapolation (39),

$$\log k'_\Phi = -S\cdot\Phi + \log k'_w, \qquad [2]$$

where Φ is the volume fraction of methanol in the eluent, S is the slope of the linear log k'_Φ–Φ plot, and log k'_w is the intercept obtained by extrapolating the plot to 100% water. The reported log k'_w values are the average of four different measurements.

Measurement of Surface Activity. Water used for buffers and solutions was passed twice through ion-exchange columns and was then distilled in glass. A 10 mM Tris buffer

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Abbreviations: BBB, blood–brain barrier; CMC, critical micelle concentration; CNS, central nervous system.

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Table 1. Molecular weights (M_r basic form) and surface areas (A_D) of a representative selection of CNS^+ and CNS^- compounds measured at pH 7.4.

Compound	No.	M_r	A_D, Å^2	Ref(s).
CNS^+				
(*R*)-apomorphine·HCl	1	267.33	ND	10
Chlorpromazine·HCl	6	318.87	42.0 ± 3.0	11
Clonidine·HCl	7	230.1	34.1 ± 2.0	12
Desipramine·HCl	8	266.39	37.2 ± 3.0	11
Doxylamine succinate	10	270.38	ND	11
cis-Flupentixol·2HCl	12	434.53	62.7 ± 2.0	11
Haloperidol·HCl	14	375.87	ND	11
Imipramine·HCl	15	280.42	67.0 ± 3.0	11
Naltrexone·HCl	18	341.41	ND	13, 14
Perphenazine·2HCl	19	403.98	57.6 ± 3.0	11
Promazine·HCl	21	284.43	57.2 ± 3.0	11
Promethazine·HCl	22	284.43	59.4 ± 3.0	11
Roxinodole methanesulfonate	23	346.48	ND	15
Tamitinol·2HCl	25	226.34	52.1 ± 3.0	16
Thiopental sodium	27	264.3	ND	11
Thioridazine·HCl	28	370.58	56.4 ± 2.0	11
Hydrophobic CNS^-				
Astemizole·2HCl	2	458.58	99.0 ± 3.0	17–21
Carebastine	4	499.66	141.0 ± 3.0	22
Domperidone·HCl	9	425.92	ND	23–25
Ebastine methanesulfonate	11	469.67	ND	22, 26
Loperamide·HCl	16	477.04	140 ± 10	23, 24, 27
Terfenadine	26	471.69	112.8 ± 3.0	17–20
Hydrophilic CNS^-				
Atenolol	3	266.34	34.1 ± 2.0	28, 29
Mequitazine·HCl	17	322.48	42.5 ± 3.0	8, 21, 30
Salbutamol hemisulfate	24	233.31	36.1 ± 2.0	31
Hydrophilic CNS^- (not surface active)				
Carmoxirol·HCl	5	374.6		32, 33
Furosemide	13	330.75		34
Pirenzepine·HCl	20	351.41		35

adjusted to the desired pH with HCl and containing 154 mM NaCl was used for all compounds except tamitinol, for which 100 mM Tris buffer/64 mM NaCl was used. Buffers were freshly prepared before use, to avoid bacterial contaminations. The monolayer trough, milled from Teflon, had a surface area of 45 cm^2 and contained 20 ml of buffer. The surface pressure was measured by the Wilhelmy method (40) by using plates cut from filter paper (Whatman no. 1) (41). Before each measurement, the trough and the filter paper were thoroughly cleaned with methanol and distilled water. The filter paper was left to equilibrate in buffer until a steady surface tension was reached. The surface tension, γ_0, of the buffer solution was set to zero and the surface pressure, π, of the drug, which is the difference between the surface tension of the pure buffer, γ_0, and the surface tension of the buffer solution containing the drug, γ, was recorded—i.e. $\pi = \gamma_0 - \gamma$.

Since some drugs could not be dissolved in water at sufficiently high concentrations (1–100 mM), methanol was used as a solvent for stock solutions. To simplify the comparison of the data, all stock solutions were made with methanol, independent of the water solubility of the respective compounds. As methanol itself exhibits some surface activity, the experimental π–log c plots were corrected for the effect of methanol as follows. According to the thermodynamics of surfaces, the variation of the surface tension, γ, is given by

$$-d\gamma = \Sigma \Gamma_i d\mu_i, \quad [3]$$

where $\Gamma_i = n_i^s/A$ is the surface excess of component i and n_i^s is the molar excess amount of compound i accumulated at the surface area A. If we neglect activity coefficients, the variation of the chemical potential, μ_i, can be written as

$$d\mu_i = RT\, d \ln C_i, \quad [4]$$

where RT is the thermal energy. In the present study both the drug concentration, C_D, and the methanol concentration, C_M, are varied and hence the change in surface tension is

$$-d\gamma = RT[(\Gamma_D\, d \ln C_D) + (\Gamma_M\, d \ln C_M)]. \quad [5]$$

In the absence of specific drug-methanol interactions Eq. 5 shows the additivity of the action of drug and methanol on the surface tension. The π–log C plot of methanol was thus measured independently and was then subtracted from the π–log C plot of the drug injected from a methanolic stock solution. The corrected π–log C plots were in good agreement with π–log C plots measured in purely aqueous solutions.

The solution was stirred with a tiny magnet and small aliquots of the drug stock solution were added with a microsyringe; the surface pressure was monitored until equilibrium was reached. For most drugs the equilibration times were in the range 15–20 min. Identical measurement times were chosen for the drug solution and the methanol control measurement in order to avoid concentration differences due to methanol evaporation. The final amount of methanol added was generally about 1 ml. Measurements were performed at 21 ± 1°C. Generally three measurements were made with the same substance under the same conditions. The reproducibility of the measurements was typically within 5%.

RESULTS

Lipophilicity Parameters Determined by Polycratic Reversed-Phase HPLC. Fig. 1 shows the lipophilicity parameters (log k'_w) of 28 drugs in the order of increasing lipophilicity as determined by polycratic reversed-phase HPLC. Also indicated is the CNS availability: CNS^- compounds are represented by solid bars, and CNS^+ compounds by hatched bars. In contrast to the prevailing opinion (9) an appreciable fraction of the CNS^- compounds exhibit high lipophilicity. Conversely, CNS^+ compounds may also be found at the low lipophilicity end of Fig. 1. Thus, the measurement of lipophilicity constants alone is not sufficient for a satisfactory prediction of the brain penetration tendency of these compounds.

Gibbs Adsorption Isotherm. Most drug molecules are amphiphilic substances due to the segregation of hydrophilic and hydrophobic groups in the same molecule. We have therefore considered whether parameters derived from surface activity measurements of drugs correlate with their CNS permeation tendency and have measured the surface pressure π as a function of drug concentration for all compounds listed in Fig. 1.

Fig. 2 shows a comparison of corrected π–log C plots of four phenothiazine analogues—perfenazine, chlorpromazine, prometazine, and mequitazine. Closer inspection of Fig. 2 reveals three parameters which are characteristic of the drug properties: (*i*) the concentration of surface activity onset, C_o, (*ii*) the constant slope of the π–log C curve reached after an initial gradual increase of the surface pressure, π, with log C, and (*iii*) the critical micelle concentration (CMC), at which the surface pressure reaches a limiting value. Unlike soap-like micelles, for which a plateau region is observed over an extended concentration range, compounds containing tertiary amines often show a surface pressure decrease soon after reaching the CMC, due to solubility limitations (42). The instability of the plateau region upon further in-

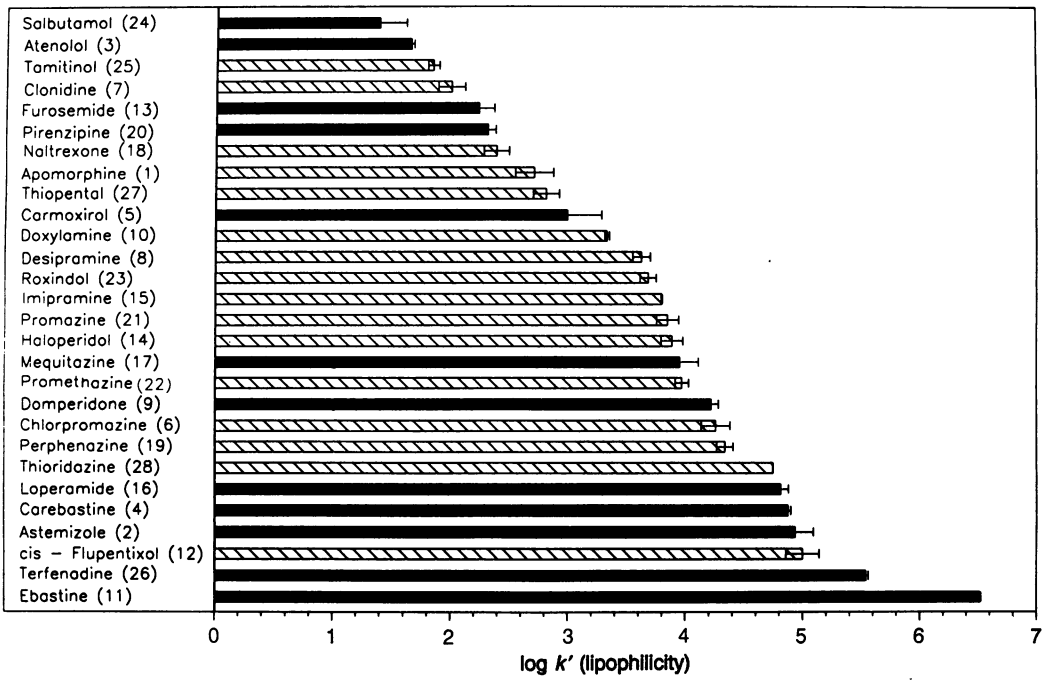

FIG. 1. Twenty-eight drugs (numbered as in Table 1) are listed in order of increasing lipophilicity. CNS^+ compounds are indicated by hatched bars, and CNS^- compounds by black bars. The lipophilicity parameters were determined by polycratic HPLC.

crease in concentration could be due to the deprotonation of the tertiary amino groups as a consequence of micelle formation. For the present compounds solubility limitations are reached at concentrations approximately twice as large as the highest concentrations included. Fig. 2 shows that the C_o values increase in the order perphenazine < chlorpromazine < prometazine < mequitazine, reflecting the order of decreasing hydrophobicity (Fig. 1). The CMC values parallel approximately the C_o values, but the differences between the four compounds are not so clear-cut.

C_o–CMC Plot. The comparison of a large number of π–log C measurements lead us to the conclusion that two parameters, C_o and CMC, depend critically on even small changes in the molecular structure. Indeed, it is the combination of these two parameters which allows a classification of the various surface active drugs according to their hydrophobicity and their ability to reach the CNS. This is demonstrated when the C_o values of all drugs investigated are plotted versus the corresponding CMC values measured either at pH 7.4 (Fig. 3*A*) or at pH 8 and 6.8, respectively (Fig. 3*B*).

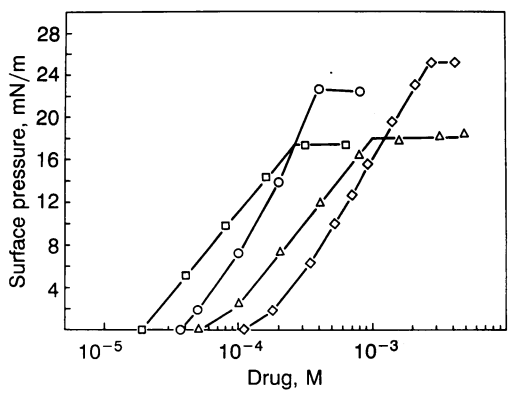

FIG. 2. The π–log C plots of perphenazine (□), chlorpromazine (○), promethazine (△), and mequitazine (◇) injected as methanolic stock solutions into Tris, pH 7.4/154 mM NaCl and corrected for the surface pressure of methanol.

Studies at pH 7.4 are pertinent to the physiological conditions in blood. Measurements at pH 8.0 for cationic drugs and at pH 6.8 for anionic drugs are relevant for the situation at the membrane surface. The rationale for the latter measurements is as follows. The penetration of a charged molecule into a nonpolar environment is energetically unfavorable and the molecule will reduce its charge, provided the pK of the charged residue is 7–8. For tertiary amines, the apparent pK shift, ΔpK, is about +0.6 (43); for the anionic thiopental, $\Delta pK \approx -0.6$ was assumed. By correcting for these membrane-induced pK shifts in appropriate buffers Fig. 3*B* approaches more closely the charge conditions of drugs in the biological membrane. For most substances the difference between the two pH values is small, but in some cases the shift can be quite dramatic (e.g., compound 16).

Charge conditions of zwitterionic drugs in the membrane cannot be simulated by a simple pH change and therefore carebastine was not included in Fig. 3*B*.

Three CNS^- compounds have to be mentioned separately—carmoxirol, furosemide, and pirenzepine. These compounds are relatively hydrophilic, with C_o values > 0.1 mM. They are, however, barely surface active, as their solubility limits almost coincide with their C_o values.

Surface Area of Drug Molecules at the Air/Water Interface. The surface excess, Γ_D, of drug molecules was estimated from the slope of the π–log C plot according to

$$\Gamma_D = (1/RT)(d\pi/d \ln C_D). \quad [6]$$

Knowledge of Γ_D allows in turn the evaluation of the area, A_D, per drug molecule at the air/water interface,

$$A_D = 1/N_A\Gamma_D, \quad [7]$$

where N_A is the Avogadro number. Compounds with low pK values which exhibit nonlinear π–log C plots were not evaluated. Numerical values are summarized in Table 1. The average surface area for CNS^+ molecules was $A_D = 52.6 \pm 3.0$ Å^2, and that for hydrophobic CNS^- compounds was $A_D = 123.2 \pm 3.0$ Å^2. The average surface area of hydrophilic CNS^- compounds is similar to those of CNS^+ compound or even slightly smaller.

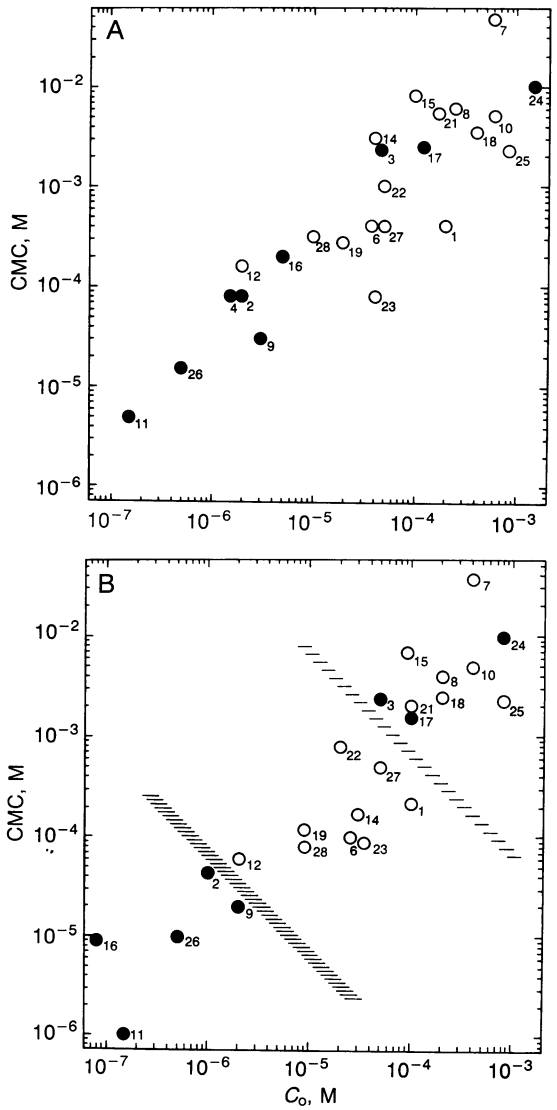

FIG. 3. CMC plotted as a function of the concentration of surface-activity onset, C_o, at pH 7.4 (*A*) and at pH 8.0 for cationic and pH 6.8 for anionic compounds (*B*). All three buffers contained 154 mM NaCl. ○, CNS^+; ●, CNS^- compounds. Hatched lines in *B* indicate the three hydrophobicity regions discussed in the text. For assignment of compounds see Table 1.

DISCUSSION

This study suggests a way to predict the potential of drugs for crossing the BBB. It is based on the measurement of the surface activity and as such takes into account the molecular properties of both hydrophobic and charged residues of the molecule of interest. The method is at least as simple as reversed-phase HPLC and is thus suited for the screening of a large number of substances, since a typical experiment does not take more than 4–5 hr.

Three parameters characterizing the surface activity of the drugs were tested for their correlation with the BBB permeability of the drug investigated. C_o appears to be related to the hydrophobicity of the drugs but does not exactly parallel the chromatographically determined hydrophobicity scale. Correlating C_o with the BBB permeability properties demonstrates that already C_o alone allows a better differentiation between CNS^+ and CNS^- compounds than does the conventional hydrophobicity scale of Fig. 1. A thermodynamic interpretation of the C_o parameter is not available, but a good correlation between C_o and the constants for binding to phospholipid model membranes has been observed for neuropeptides (44, 45) and some of the present drugs (A. Frentzel and A.S., unpublished work).

The CMC parameter alone was not found to be useful in predicting the BBB permeability. However, a most conspicuous result was obtained by combining C_o and CMC in two-dimensional plots. Fig. 3*A* refers to pH 7.4 and describes the situation in the bloodstream; Fig. 3*B*, measured at pH 8.0 (cationic drugs) and pH 6.8 (anionic drugs), simulates the behavior of drugs after entering a hydrophobic membrane. Both diagrams can be divided into three regions: (*i*) a region of CNS^- drugs constituted by very hydrophobic compounds, (*ii*) a region of less hydrophobic compounds which penetrate easily into the CNS, and (*iii*) a region of more hydrophilic compounds which become CNS^+ only when employed at high concentrations. Fig. 3 comprises 28 drugs and we suggest that they may be used as standards to predict the CNS penetration power of unknown drugs. A comparison of the lipophilicity coefficients (Fig. 1) with the C_o–CMC plot shows that the latter allows a more reliable prediction of the BBB permeability properties of a given drug.

An additional criterion which may be helpful in differentiating between CNS^+ and CNS^- drugs is the surface area, A_D. Inspection of Table 1 demonstrates that those hydrophobic CNS^- compounds have distinctly larger areas than the CNS^+ compounds. This is particularly obvious for loperamide. From clinical studies loperamide is known to be CNS^-, but nevertheless it appears in the region of CNS^+ compounds of Fig. 3*A*. However, if we use the A_D as an additional exclusion criterion, loperamide must be grouped together with CNS^- compounds. Likewise, a shift in pH (Fig. 3*B*) moves loperamide into the CNS^- region.

The latter pH effect deserves further attention, since it is intimately related to pK shifts experienced by amphiphilic drugs upon binding to lipid membranes. Most of the drugs listed in Fig. 1 carry at least one tertiary amino group, the average pK value of which is 8.5 (46). When dissolved in blood (pH 7.4), the amino group is almost fully protonated. However, when inserted into the headgroup region of the endothelial membrane facing the blood, which has a low content of negatively charged lipids (47, 48), the tertiary ammonium ions are destabilized, leading to an apparent pK decrease of at least 0.6 (43, 49–51). It is the uncharged form that can then diffuse through the membrane but becomes reprotonated in the brain fluid, after leaving the membrane. The deprotonation in the hydrophobic membrane is anticipated in Fig. 3*B* by employing buffers of increased pH (cationic drugs) or decreased pH (anionic drugs).

Why are very hydrophobic compounds hindered from reaching the CNS, since the low C_o values indicate a strong tendency to bind to lipid membranes? Apparently, binding is not restricted to lipids but can take place at any other amphiphilic site—e.g., proteins. A high tendency to bind plasma proteins has indeed been reported for astemizole, terfenadine, and loperamide (up to 97% in protein-bound form) (11), leading to a low concentration of these drugs in the endothelial membrane. Another reason might be the hydrophobic "trapping" effect of the membrane interior. The drugs could simply accumulate in the hydrophobic interior of the bilayer and might not be able to reprotonate at the CNS side of the membrane in order to leave for the brain fluid.

Compounds in group *ii* are less hydrophobic and have smaller cross-sectional areas at the air/water interface. They bind readily to lipid membranes and their pK values are in general high enough to guarantee a reprotonation at the CNS side of the membrane.

The relatively hydrophilic compounds in group *iii* cross the BBB only if applied in sufficiently high concentrations. By increasing the concentration of the drug in the blood the equilibrium is shifted from the free drug toward the mem-

Table 2. Phenothiazines and a related compound, *cis*-flupentixol, listed in the order of increasing hydrophilicity

Compound	No.	Mode of Action	Dose, mg/day
cis-Flupentixol	12	Antipsychotic	6–18
Thioridazine	28	Antipsychotic Sedative	20–300
Perphenazine	19	Antipsychotic	12–24
Chlorpromazine	6	Antipsychotic Antiemetic Antihistaminic	75–300
Promethazine	22	Antiemetic Antihistaminic CNS depressant	25–75
Mequitazine	17	Antihistaminic	10
Promazine	21	Antipsychotic	400–800

brane-bound drug. Therefore therapeutically active concentrations of centrally acting drugs must be increased with increasing hydrophilicity (increasing C_0 values at pH 7.4) within a homologous series provided that a comparable affinity for the respective receptor exists. This is exemplified in Table 2 for a number of phenothiazines and a related thioxantine. The compounds are listed in the order of increasing hydrophilicity together with their daily doses (11), which increase in the same order for the antipsychotic compounds. From Table 2 it is evident that mequitazine, which produces maximal occupation of lung receptors at a much lower concentration than needed to cross the BBB, appears as a CNS^- compound and has central effects only if applied at higher concentrations (30).

Atenolol, which appears to be incompletely absorbed from the gastrointestinal tract (11), and salbutamol, which is the most hydrophilic compound measured, might appear as CNS^- compounds for the same reasons as mequitazine. Clonidine, which is also a very hydrophilic compound, seems to be an exception, as it reaches the brain even if applied in small concentration (11).

Three compounds listed as CNS^- in Fig. 1—carmoxirol, furosemide, and pirenzepine—were not included in Fig. 3 simply because they did not exhibit any measurable surface activity. It is evident that these compounds should not enter the brain by passive diffusion.

We have shown that the surface activity measurements (π-log C plot) allow one to predict the ability of a drug to cross the BBB with much higher reliability than the conventional partitioning methods. Taking the C_0–CMC plot in Fig. 3*B*, established with compounds of known ability to cross the BBB, as a reference should allow one to predict whether or not a compound with unknown characteristics enters the brain. Compounds which do not cross the BBB (CNS^-) are either (*i*) not surface active, (*ii*) very hydrophobic, with low C_0 and CMC values and large cross-sectional areas at the air/water interface (much larger than that of a lipid molecule), or (*iii*) relatively hydrophilic with high C_0 and CMC values especially when applied at low concentrations. Compounds which do cross the BBB (CNS^+) exhibit intermediate C_0 and CMC values and have cross-sectional areas which are smaller than that of a lipid molecule.

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