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Original article

The factors that influence permeation across the blood–brain barrier

Michael H. Abraham

Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK

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Abstract

Using data on perfusion from saline at pH 7.4 taken from the literature, it is shown that logarithms of PS-products, log PS, can satisfactorily be correlated with the five Abraham descriptors for 30 neutral compounds. The equation has R^2 = 0.870 and *S.D.* = 0.52, and shows that molecular size leads to an increase in permeation rate, and dipolarity/polarizability, hydrogen bond acidity and hydrogen bond basicity all decrease the rate of permeation. There are difficulties with acids and bases that are partially ionised at pH 7.4 because it is not possible to assess the rate of permeation of the resulting ionic species with respect to the neutral species. The same problem will arise in the analysis of perfusion from plasma or blood.

Keywords: Brain permeation; Permeability-surface area product; Hydrogen-bonding; LFER; Solute descriptors; Acids and bases

1. Introduction

There are a number of measures of 'brain uptake' or 'brain penetration' that are used to assess the ability of drugs to cross the blood–brain (BB) barrier. Hansch et al. [1] noted that the hypnotic activity of a series of drugs reached a maximum when the value of log *P*(oct) was around 2.0, and this seems to have led to the well-known 'rule-of-two' [2]. *P*(oct) is the water–octanol partition coefficient. Although hypnotic activity implies that a drug must have penetrated the BB barrier, lack of activity cannot be taken to show that a drug has not penetrated the BB barrier. A more useful measure is the brain uptake index (BUI) [3,4] that provides a rank order of ability to permeate the BB barrier [5]. An even more quantitative indication is the permeability, as obtained by Ohno et al. [6] and Rapoport et al. [7]. Later workers [8–11] used the in situ perfusion technique in which the rate of transfer from saline or blood to brain is expressed as a permeability-surface area product, PS in units of $cm^3\ s^{-1}\ g^{-1}$, or as a permeability coefficient, PC in units of $cm\ s^{-1}$, if the surface area of capillaries is known.

Several workers have related log PC or log PS to log *P*(oct) or to a function of *P*(oct) and molecular weight, MW, that takes the form of log [*P*(oct)*(MW)$^{-0.5}$]; the significance of the molecular weight function is none too clear. Nevertheless, several such correlations for limited data sets have been reported [6,7,10,12,13]. Begley [14] collected a number of log PC values and showed that there was a rather scattered plot against log *P*(oct) for 24 compounds. Several other drugs were considerable outliers and included substrates for the *P*-glycoprotein efflux pump as well as those transported by carrier mediated mechanisms. Bodor and Buchwald [15] carried out a similar analysis and 'omitting the obvious outliers' obtained the correlation equation shown as Eq. (1). However, the PC values used appear to include permeation from both saline and plasma (corrected for protein binding), and for some compounds log *D*(oct) at pH 7.4 was used instead of log *P*(oct). Unfortunately, no numerical details are given

(1) Log PC = –5.102 + 0.566 log *P*(oct)

$N = 58$, $R^2 = 0.803$, S.D. = 0.62, $F = 226.8$.

In Eq. (1) and elsewhere, *N* is the number of data points, R^2 is the variance, S.D. is the standard deviation and *F* is the *F*-statistic. Gratton et al. [16] determined PS-products for 18 compounds, using saline at pH 7.4 as the perfusate, and obtained a reasonable correlation with Abraham's solvation parameters; however, the number of compounds is too small to obtain any definitive correlation.

By comparison to the above correlations of PC or PS values, there are now a large number of in silico correlations of blood–brain distribution, as detailed by Abraham et al. [17]. Indeed, it is clear that predictions of log (blood–brain

E-mail address: m.h.abraham@ucl.ac.uk (M.H. Abraham).

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distribution) can be made with S.D. of around 0.37 log units—not far from the experimental error in the determinations. Since the work of Gratton et al. [16], Hider et al. [18] have determined further PS values by the same method and so it seemed timely to collect the available PS values and to re-analyse them by the method of Abraham.

2. Chemistry

The PS values used are mainly based on those determined by Gratton et al. [16] for a variety of compounds and by Hider et al. [18] for a number of hydroxypyridinones. They refer to perfusion from protein-free saline, buffered to pH 7.4. In addition, Pardridge and Mietus [12] have obtained PC and PS values for six steroids, Chikhale et al. [19] have obtained PC values for seven peptides and have determined the PC values that relate to intrinsic passive diffusion only. These were converted into PS values using a capillary surface area of 130 $cm^2\ g^{-1}$. A PS value for glycerol is also available [6], all determinations using saline as the perfusate. The compounds are given in Table 1. The hydroxypyridinones are listed as 'CP' numbers in exactly the same way as Hider et al. [18] who give the various structures. The peptides studied by Chikhale et al. [19] are again listed as in the same way as before. Although Gratton et al. [16] gave a PS value for sucrose, this has not been used as it is a huge outlier. Gratton et al. gave PS values for a number of compounds (nos. 12–18, in parenthesis, in Table 1) that are proton acids or proton bases, and which will be partially ionised in saline at pH 7.4; these values have also not been used, and will be discussed later. There are left 30 log PS values for neutral compounds, i.e. compounds that are not ionised at pH 7.4, for analysis.

The general solvation equation employed to correlate the log PS values is the linear free energy relationship, LFER, as set out in several publications [20–24]

(2) $SP = c + e{\cdot}E + s{\cdot}S + a{\cdot}A + b{\cdot}B + v{\cdot}V$.

Here SP is a set of solute properties in a given system, in the present case log PS. The independent variables are solute descriptors as follows: E is an excess molar refraction in units of ($cm^3\ mol^{-1}/10$), S is the dipolarity/polarizability, A and B are the hydrogen bond acidity and basicity, respectively, and V is the solute McGowan volume in units of ($cm^3\ mol^{-1}/100$). The required descriptors are given in Table 1. Those for the peptides are taken from Abraham et al. [25] who also list the compound structures.

3. Results

Application of Eq. (2) to the 30 log PS values for neutral compounds in Table 1 leads to Eq. (3), where the S.D. values for the coefficients are given in parentheses. The term in $e{\cdot}E$ is not significant, but is retained for purposes of comparison. If this term is left out, then Eq. (4) results.

(3) Log PS = –0.639(0.408) + 0.312(0.515)E – 1.009(0.158)S – 1.895(0.385)A – 1.636(0.410)B + 1.709(0.392)V

$N = 30$, $R^2 = 0.870$, S.D. = 0.52, $F = 32.2$.

(4) Log PS = –0.716(0.383) – 0.974(0.146)S – 1.802(0.349)A – 1.603(0.401)B + 1.893(0.245)V

$N = 30$, $R^2 = 0.868$, $S = 0.52$, $F = 41.2$.

Eq. (3) is statistically quite reasonable; S.D. is only 0.52 log unit, over a range of log PS values covering nearly five log units. Unfortunately, there are not enough data points to be able to determine the predictive power through division into a training set and a test set. The calculated log PS values in Eq. (3) are given in Table 1, and a plot of observed vs. calculated values is shown in Fig. 1.

Compounds that are proton acids or proton bases, and hence partially ionised in saline at pH 7.4 have not been included in the above analysis. Knowing the compound pK_a, it is easy to calculate the fraction that exists as the neutral form, FN, and the fraction that exists in the ionised form, FI. From the value of FN, it is then possible to determine the log PS value that corresponds to the neutral form, on the assumption that the ionised form does not undergo permeation at all. This log PS value might be regarded as a 'fully corrected' value and is denoted as log PS (corr). However, the log PS value calculated from Eq. (3) also corresponds to the value for the neutral form, because Eq. (3) contains only unionised compounds, and because the descriptors in Table 1 are for the unionised compounds. These values are the calculated values in Table 1.

4. Discussion

Although the statistics of Eqs. (3) and (4) appear to be quite good, there is a difficulty in that significant cross-correlations exist between the descriptors. Values of R^2 between pairs of descriptors are: *E/S* 0.90, *E/A* 0.00, *E/B* 0.80, *E/V* 0.94, *S/A* 0.01, *S/B* 0.78, *S/V* 0.86, *A/B* 0.04, *A/V* 0.05, and *B/V* 0.81. A partial least squares analysis with five components yields exactly the same coefficients as those in Eq. (3), as required. Analyses with four components or three components yield coefficients in reasonable agreement with those in Eq. (3) which suggests that Eqs. (3) and (4) are quite soundly based.

Eq. (3) is important, that it shows the main factors that influence permeation across the BB barrier. Compound polarity of any sort, that is dipolarity/polarizability, hydrogen bond acidity and hydrogen bond basicity leads to a decrease in the rate of permeation, and compound size as measured by the McGowan volume, *V,* leads to an increase in permeation rate. Although different to the log PS equation derived by Gratton et al. [16], Eq. (3) leads to the same qualitative results. However, the new Eq. (3) includes enough data to speculate on possible cut-off effects due to size. It may be expected that as the compound size increases, there will be a

Table 1
Compound descriptors, observed log PS, calculated log PS in Eq. (3), and log *P*(oct)

Compound	*E*	*S*	*A*	*B*	*V*	Log PS Observe [a]	Log PS Calculated [b]	Log *P*(oct)
CP20 [c]	0.977	1.31	0.10	1.21	1.0745	–1.89	–1.99	–0.77
CP21	0.932	1.31	0.10	1.21	1.2154	–1.48	–1.76	–0.31
CP24	0.918	1.31	0.10	1.21	1.4972	–0.64	–1.29	0.70
CP29	0.915	1.31	0.10	1.21	1.6381	–0.38	–1.05	1.24
CP25	0.905	1.31	0.10	1.21	1.7790	–0.36	–0.81	1.90
CP94	0.894	1.31	0.10	1.21	1.3563	–1.03	–1.53	0.23
Erythritol [d]	0.620	1.60	0.48	1.39	0.9070	–4.57	–3.69	–2.29
Urea	0.501	1.49	0.83	0.84	0.4646	–3.79	–4.14	–2.11
Ethylene glycol	0.404	0.90	0.58	0.78	0.5078	–2.99	–2.93	–1.36
Thiourea	0.840	0.82	0.77	0.87	0.5696	–3.36	–3.11	–1.02
Propan-2-ol	0.212	0.36	0.33	0.56	0.5900	–1.66	–1.47	0.05
Ethanol	0.246	0.42	0.37	0.48	0.4491	–1.52	–1.71	–0.30
Antipyrine	1.320	1.50	0.00	1.48	1.5502	–2.00	–1.51	0.23
Mannitol	0.836	2.83	0.95	1.65	1.3062	–5.01	–5.50	–2.98
Estradiol	1.800	1.77	0.86	1.10	2.1988	–0.83	–1.54	4.01
Thymine	0.800	1.00	0.44	1.03	0.8925	–3.35	–2.39	–0.62
Corticosterone [e]	1.860	3.43	0.40	1.63	2.7389	–2.29	–2.26	1.94
Aldosterone	2.010	3.47	0.40	1.90	2.6890	–3.46	–2.79	1.08
Hydrocortisone	2.030	3.49	0.71	1.90	2.7976	–3.85	–3.20	1.55
Estradiol	1.800	1.77	0.86	1.10	2.1988	–1.74	–1.54	4.01
Testosterone	1.540	2.59	0.32	1.19	2.3827	–1.72	–1.25	3.31
Progesterone	1.450	3.29	0.00	1.14	2.6215	–1.74	–0.89	3.70
Glycerol [f]	0.512	0.90	0.70	1.14	0.7074	–2.98	–3.37	–1.76
$AcFNH_2$ (I) [g]	1.453	3.90	0.65	0.89	1.6519	–3.80	–3.99	0.05
$AcFFNH_2$ (II)	2.466	5.20	0.67	1.63	2.7979	–4.28	–4.27	1.19
$AcFFFNH_2$ (III)	3.479	6.60	0.64	2.27	3.9439	–4.39	–4.40	2.30
$AcFF(NMeF)NH_2$ (IV)	3.441	6.45	0.50	2.37	4.0848	–3.74	–3.92	2.63
$AcF(NMeF)_2NH_2$ (V)	3.403	6.60	0.16	2.48	4.2257	–3.31	–3.38	2.53
$Ac(NMeF)_3NH2$ (VI)	3.365	6.50	0.00	2.50	4.3666	–2.47	–2.78	2.92
$Ac(NMeF)_3NHMe$ (VII)	3.302	6.10	0.00	2.55	4.5075	–2.06	–2.23	3.24
Sucrose [d,h]	1.970	2.50	2.10	3.00	2.2279	–5.30	–7.59	–3.34
22001 (12) [d,i]	1.710	2.46	0.34	1.25	1.4122	–2.92	–2.87	1.55
12002 (13)	2.585	2.50	0.71	1.20	2.3595	–3.29	–1.64	3.80
11003 (14)	2.050	3.80	0.48	2.25	3.9242	–1.53	–1.71	3.63
95005 (15)	1.230	2.25	0.50	1.44	2.0765	–2.61	–2.28	1.26
26006 (16)	3.110	4.50	0.37	2.93	4.8010	–3.31	–1.48	3.90
13007 (17)	0.848	0.98	0.16	0.63	1.1507	–1.57	–0.74	2.19
Propranolol (18)	1.880	1.43	0.17	1.42	2.1480	–1.02	–0.47	2.98

[a] Observed log PS values in $cm^3\ s^{-1}\ g^{-1}$.
[b] In Eq. (3).
[c] Ref. [18].
[d] Ref. [16].
[e] Ref. [12].
[f] Ref. [6].
[g] Ref. [19].
[h] Not used.
[i] Ref. [16].

point where the rate of permeation starts to decrease. With the 30-compound data set, this point has not been reached. Peptides VI and VII have molecular weights of 543 and 557, and both fit well to Eq. (3). Calculated and observed values of log PS are for peptide VI –2.77 and –2.47, and for peptide VII –2.23 and –2.06, respectively.

Another important use of Eq. (3) involves comparison with other equations derived from Eq. (2) in order to identify possible model processes and to obtain information on the mechanism of the permeation process. Two analytical methods for such comparison are non-linear mapping [26] as applied specifically to Eq. (2) by Valko et al. [27], Du et al. [28], and the mathematical analysis of Ishihama and Asakawa [29], again applied specifically to Eq. (2). Both of these methods require all five coefficients in Eqs. (2) and (3). That is why it is important to retain all five coefficients, even though Eq. (4) is statistically better than Eq. (3) as judged by the *F*-test.

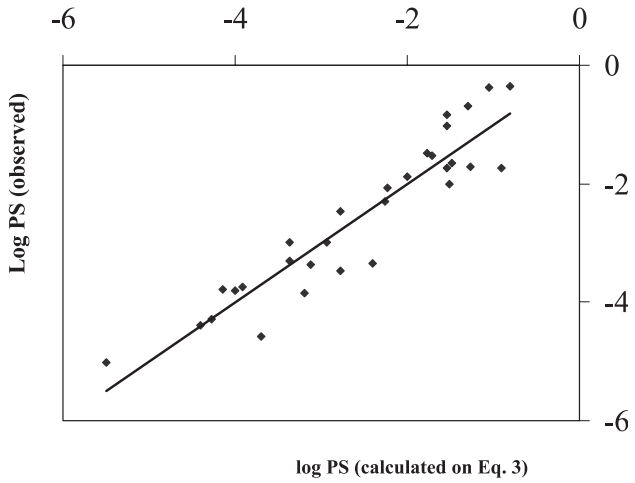

Fig. 1. A plot of log PS (observed) vs. log PS (calculated in Eq. (3)).

As pointed out in the introduction, there are several cases where reasonable plots of log PS against log P(oct) or log [P(oct) × (MW)$^{-0.5}$] have been obtained. Begley [14] commented on the rather scattered plot of log PS against log P(oct) that he obtained for 24 compounds, but the plot obtained by Bodor and Buchwald [15], and the related regression equation (1) represents a respectable correlation. For the data set consisting of perfusion from saline of 30 neutral compounds used in this work, a quite different result is obtained. The plot of log PS against log P(oct) shown in Fig. 2 is nearly random, and a regression yields R^2 = 0.146 only. There is no obvious explanation of this difference. Since the 30 compound set used in this work relates to the same type of perfusate (saline), and excludes compounds that might be ionised at pH 7.4, any connection between log PS and log P(oct) should be more evident, not less. This random scatter can hardly be due to an efflux mechanism because the peptides I–VII lie on the lower part of Fig. 2, and permeation of these compounds is known to be by simple passive transport [19].

Liu and Hider [30] found that for a series of hydroxypyridinones, a plot of log PS against the % polar surface area,

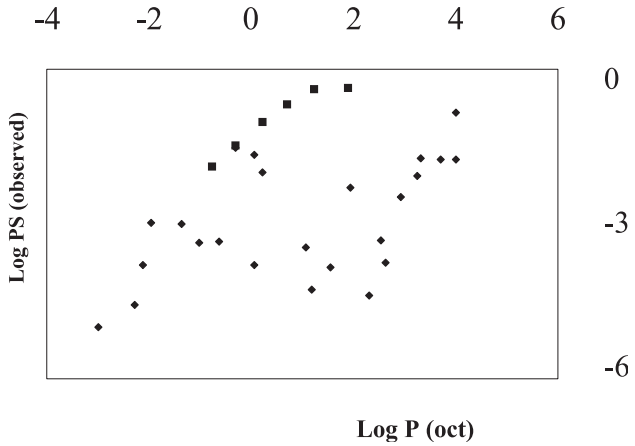

Fig. 2. A plot of log PS (observed) vs. log P(oct) for the 30 neutral compounds. (■) Compounds in the Hider (CP) set.

PSA, was a remarkable straight line. Polar surface area, non-polar surface area, NSA, and the %PSA have been calculated for the 30 compounds using PCMODEL [31]. A plot of log PS against %PSA (not shown) is almost random and yields R^2 = 0.167 for the regression. Actually, PSA itself is a better descriptor for the 30 compounds (R^2 = 0.428), and a double regression in PSA and NSA yields an overall value of 0.603 for R^2.

It appears that in only one paper dealing with brain perfusion [16] has the possibility of ionisation of proton acids and bases been mentioned. Admittedly, the problem of ionisation in blood or plasma is rendered intractable because no pK_a values in these media are available. But this is not the case in saline, where pK_a values in water can be used. Gratton et al. [16] took the view that log PS values for ionizable compounds should be corrected on the basis that only neutral species would undergo perfusion. Recent work has indicated that for rates of transfer from one phase to another, such a classical correction may not be appropriate. Wohnsland and Faller [32] carried out measurements of permeation from aqueous solution through a layer of hexadecane immobilised on a polycarbonate filter. They showed that rates of permeation of proton acids and proton bases as a function of the aqueous pH were not in accord with calculated permeation-pH profiles. Thus for the strong base, desipramine, with pK_a = 10.6, the rate of permeation at pH 7.4 was the same as at pH 9 or 10, even though at pH 7.4 the ratio of ionised to neutral species is 1.6 × 10^3. Zhao et al. [33] observed that the intestinal absorption of proton acids and proton bases could reasonably be accounted for by use of descriptors for the neutral forms and by ignoring ionisation. The effect of ionisation was very small indeed. Abraham and Martins [34] have analysed data on the rate of permeation of proton acids and bases from water through human skin. They concluded that the ionised forms of carboxylic acids permeated so slowly that permeation could be ignored, but that permeation of the ionised forms of nitrogen bases could not be ignored. Very approximately, the latter permeated at about 1/15 the rate of the corresponding neutral base.

As regards perfusion from saline, it is therefore by no means clear if the observed log PS values should be taken as they are (that is perfusion of the ionised species is the same as that of the neutral species) or if the full classical correction should be applied (that is perfusion of the ionised species is so slow as to be ignored) or if some intermediate correction should be made (that is the ionised species permeates at some fraction of the rate of permeation of the neutral species). Gratton et al. [16] measured log PS for a number of acidic and basic species, not used to obtain Eq. (3). Details of these compounds and their pK_a values are in Table 2. From the pK_a values, it is possible to calculate the fraction of neutral species present at pH 7.4, listed in Table 2 as F(N). Also given are the observed log PS values and the log PS values obtained by the full classical correction. Eq. (3) can also be used to calculate log PS; these values must be for the neutral species only, because Eq. (3) is constructed only for neutral species,

Table 2
Details of ionisation of acids and bases studied by Gratton et al. [16]

Name [a]	pK_a	Log PS			F(N)	PS (I/N)
		Observed	Full correction	Eq. (3)		
22001 (1A)	5.30	–2.92	–0.82	–2.87	0.00800	0.89
12002 (2A)	3.20	–3.29	0.91	–1.64	0.00006	0.02
Propranolol (3B)	9.40	–1.02	0.98	–0.47	0.00990	0.27
11003 (4B)	8.10	–1.53	–0.75	–1.71	0.16600	1.62
13007 (5B)	10.10	–1.57	1.13	–0.74	0.00199	0.15
95005 (6B)	7.95	–2.61	–1.95	–2.28	0.22000	0.32
26006 (7B)	8.91	–3.31	–1.79	–1.48	0.02990	0.00

[a] Numbers as in Ref. [16]. A and B denote acidic and basic compounds.

and the descriptors used are also only for the neutral species. Within the error limits of the calculation, Eq. (3) data points lie within the observed and the fully corrected values, rather nearer to the observed values. This suggests that for this very limited data set, perfusion of the ionic species is slower than that of the corresponding neutral species, but cannot be ignored. If Eq. (3) calculated log PS values are taken as estimates of permeation of the neutral species, then it is possible also to estimate the ratio of PS values for the ionic species to the neutral species denoted as PS (I/N). These are in the final column in Table 2. They cannot be accurate to more than a factor of 2 or 3, because none of them would be expected to be greater than 1. However, they indicate that permeation of ionic species must be taken into account so that a partial correction to observed PS values can be made in order to obtain PS values for the neutral species. Unfortunately, the present results are so limited and scattered that it is not possible to suggest that there might be a general value of PS (I/N) for acids or bases that could be used in the future. Until further results are available, there will remain uncertainty as to how (or if) to correct observed PS values in saline, and of course in blood, for ionisation. This leads to the difficulty of predicting permeation from saline through the BB barrier, because equations such as Eq. (3) will predict only PS values for the neutral species, and will not predict 'observed' values for species that are significantly ionised at the aqueous pH used.

An alternative interpretation is based on the concept of an unstirred water layer, or stagnant layer [35,36], adjacent to the water–BB barrier phase boundary, through which the species must diffuse. A correction for diffusion through the stagnant layer can be made, but only by carrying out experiments at different aqueous pH values [32]. Wohnsland and Faller [32] related their results to an 'apparent pK_a' value for proton acids and proton bases, but at the moment there is no way of predicting such a pK_a value. Once again the same difficulty exists as regards prediction of 'observed' values of log PS for species that are significantly ionised at the aqueous pH used.

5. Conclusions

Values of log PS for perfusion from saline at pH 7.4 can satisfactorily be correlated against the five Abraham descriptors, for 30 neutral compounds. For two proton acids and five proton bases, correlation and prediction of log PS is rendered difficult because it is not clear how well the ionised forms of the acids and bases undergo permeation. From the limited data, it seems as though the ionic forms undergo permeation less readily than the neutral forms, but any quantitative assessment must needs wait until more information is available. For permeation from blood or plasma, a similar difficulty must also exist.

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