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Permeability of the Blood–Brain Barrier: Molecular Mechanism of Transport of Drugs and Physiologically Important Compounds

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Abstract A new molecular model for the permeability of drugs and other physiologically important compounds to cross the blood–brain barrier has been developed. Permeability (log *PS*) is dependant on desolvation, lipophilicity, molecular volume and dipole moment. Previous models for BBB permeability have not considered desolvation and dipole moment as critical factors. The model applies to passive diffusion processes, and some facilitated diffusion processes. Passive permeability models may not apply to active transport processes, where complex membrane protein binding processes (e.g. stereoselectivity) are involved. Model phosphatidylcholine lipid bilayer membranes have been used to evaluate how charged or polar neutral compounds can interact through their molecular dipoles with the cell membrane to induce electromechanical changes in the cell membrane which facilitate permeation. The free energy of solvation in *n*-octanol has been shown to be a good measure of membrane lipophilicity by calculating the solvation free energy of a model PC lipid membrane in a series of closely related alcohols. The passive diffusion model for alcohols correlates with the known modulation of membrane bilayers which showed a size-dependent "cut-off" point in potency. For most drugs and related molecules, the neutral species are the permeating species.

Keywords Permeability · Blood–brain barrier · Quantum mechanics · Molecular mechanism · Quantitative models

Abbreviations

BBB	Blood–brain barrier
CNS	Central nervous system
Log *PS*	Log value of permeability surface area
QSAR	Quantitative structure–activity relationships
PC	Phosphatidylcholine
DPPC	Dipalmitotylphosphatidylcholine
DPHYPC	Diphytanoylphosphatidylcholine
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine
DLPC	Dilauroyl phosphatidylcholine
DOPSE	1,2-Dioleoyl phosphatidylserine
QM	Quantum mechanics
PSA	Molecular polar surface area
ΔG_{water}	Water desolvation free energy
$\Delta G_{octanol}$	Solvation free energy in *n*-octanol, or lipophilicity
V	Molecular volume in *n*-octanol
D	Dipole moment in water
SEE	Standard error of the estimate (log *PS*)
R^2	Regression correlation coefficient

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Introduction

One of the biggest challenges of developing therapeutic agents for the treatment of neurodegenerative disorders is the blood–brain barrier (BBB). Effective drug delivery means that the BBB needs to be circumvented to achieve adequate drug doses in the brain. It has been noted that 98 % of drugs fail in clinical trials due to inadequate BBB permeability (Pardridge 2007).

The BBB plays an important role in the homoeostasis, or maintenance of the central nervous system CNS, by controlling the movement of nutrients and toxins to and from

the CNS. Drugs that have non-CNS targets need to have characteristics that prevent transport across the BBB to avoid unwanted side effects.

The BBB is a highly selective permeable cellular phospholipid protein bilayer barrier that separates the circulating blood from the brain extracellular fluid in the CNS. The BBB is composed of capillary endothelial cells, which are connected by tight junctions with an extremely high electrical resistivity of at least 0.1 Ω m. The BBB also includes a thick basement membrane and astrocytic endfeet. The BBB allows the passage of water, some gases, and lipid soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial to neural function. The BBB also protects the brain from many common bacterial infections. Antibodies are too large to cross the BBB, and only certain antibiotics are able to pass.

Compounds cross the BBB by a variety of mechanisms (Mangas-Sanjuan et al. 2010; Nau et al. 2010; Gabathuler 2010; Banks 2009; Pajouhesh and Lenz 2005):

1. *Trans-membrane or trans-cellular passive non-saturable diffusion*: usually molecules with high lipophilicity and low molecular size can passively diffuse across the BBB in the direction of the concentration gradient, without the input of energy. Paracellular diffusion is usually negligible because of the tight junctions between cells.
2. *Active saturable transporters* are integral membrane proteins (ATP dependent or ATP independent) which can transport drugs across the BBB against the concentration gradient. There are two types of transporters: (a) carrier-mediated transporters, and (b) active efflux transporters (e.g. *p*-glycogen) which carry drugs and other compounds out of the brain. There are two classes of membrane transport proteins: carrier proteins, which carry specific molecules across, and channel proteins, which form a narrow pore through which ions can pass. Channel proteins carry out passive transport, in which ions travel spontaneously down their gradients. Some carrier proteins mediate passive transport (also called *facilitated diffusion*), while others can be coupled to a source of energy to carry out active transport, in which a molecule is transported against its concentration gradient. *Facilitated diffusion* is a process of spontaneous passive transport which does not require ATP energy, and differs from passive diffusion in relying on binding between the drug and carrier protein or membrane-embedded channel, and it is a saturable process which is more reliant on temperature-dependent binding processes than passive diffusion. The main role of the drug transporters is carrying the drugs and other xenobiotics into and out of the brain, and they are integral to other cell processes such as inflammation, differentiation of immune cells, cell detoxification, lipid trafficking, hormone secretion and development of stem cells.
3. *Endocytosis and exocytosis* whereby substances (proteins, etc.) are engulfed by the membrane and pass through the cell by vesicles and released on the other side.
4. *Extracellular pathways*.

Important Molecular Properties Associated with BBB Permeability

Pajouhesh and Lenz (2005) have reviewed various retrospective classification databases in the literature to determine the common attributes and their ranges that facilitate BBB permeability:

1. Experimental in vivo measures of permeability: log BB (which is a steady state equilibrium measure of the drug partitioning in the blood or brain) or log *PS* (obtained from in situ brain perfusion studies, usually using rats, is a kinetic rate measure of the volume cleared per unit time). An effective permeability $>1 \times 10^{-6}$ cm/s is considered a lower limit.
2. Lipophilicity has a positive correlation with ability to cross BBB: usually log $P_{(o/w)}$ for neutral compounds, with a minimal hydrophobicity (*C*log $p > 5$).
3. Hydrogen bonding or polarity has a negative correlation with ability to cross BBB: indicators include Abraham coefficients, or the number of acidic and basic atoms, or number of H-bond donor atoms <3, and number of H-bond acceptor atoms <7.
4. Molecular weight <450, though there are exceptions (Banks 2009)
5. Molecular topological polar surface area (TPSA): <60–70 $Å^2$
6. Molecular shape: spherical shape preferred over rod shape, increased branching shows negative correlation with ability to cross BBB: McGowan characteristic volume for molecular size
7. Molecular flexibility has a positive correlation with ability to cross BBB, with the number of rotatable bonds being <8
8. The concentration of uncharged chemical species in water at the physiological pH level is critical, with the estimated pK_a range for BBB permeability being 4–10 (Fischer et al. 1998) or 7.5–10.5 (Pajouhesh and Lenz 2005) The presence of a positive charge at pH 7–8, or compounds with a tertiary N atom, tends to enhance BBB permeability (Goodwin and Clark

2005). Strong acids, including carboxylic acids, and bases are generally not easily transported across the BBB.

9. Metabolic stability with >80 % remaining after 1 h is desirable, since a high metabolic rate would remove the drug rapidly from the blood plasma.
10. Not being a high-affinity serum albumin ligand ($K_d < 10$ μM), since this would decrease the effective concentration of the drug in blood plasma.

Statistical (multiple linear regression) quantitative structure–activity relationships (QSAR) models: The major descriptors (Pajouhesh and Lenz 2005; Fischer et al. 1998; Jouyban and Soltani 2012; Vilar et al. 2010; Goodwin and Clark 2005; Abraham et al. 1997; Mehdipour and Hamidi 2009; Kaznessis 2005; Garg et al. 2008) found to be important in QSAR models (which predominantly seek correlations with log BB) are as follows:

1. Lipophilicity, usually expressed as *C*log *P*, has been found to be a critical factor relating to permeability. *C*log *P* has a median value of 2.5 for successful CNS drugs. Alternatively, log *D* should be between 0 and 3 for smaller compounds.
2. It has been suggested that the molecular weight (MW) should be below 400–600 for successful CNS drugs (lower than the MW of drugs undergoing oral absorption). The mean MW for marketed CNS drugs is 310, whereas the median for orally active drugs is 377.
3. All the QSAR models include hydrogen bonding, either as polarity, polar surface area (PSA), hydrogen-bond donor or acceptor coefficients (Abraham coefficients), or counting heteroatoms (O, N atoms) capable of hydrogen bonding. Generally, CNS drugs tend to have lower PSA values than other drugs, usually falling within the range 60–90 $Å^2$. There is also a trade-off relationship between polarity or PSA of a molecule and lipophilicity for larger organic compounds, where the polar component is counter balanced by the hydrophobic component of the molecule.
4. The consistent finding in QSAR modelling shows that lipophilicity is positively correlated, PSA is negatively correlated, hydrogen bonding is negatively correlated and molecular size (or molecular weight) is negatively correlated to permeability (Pajouhesh and Lenz 2005; Fischer et al. 1998; Jouyban and Soltani 2012; Vilar et al. 2010; Goodwin and Clark 2005; Abraham et al. 1997; Mehdipour and Hamidi 2009; Kaznessis 2005; Garg et al. 2008). There is some evidence that molecular volume might show a parabolic relationship to permeability, since a smaller volume positively increases diffusion, but a larger molecular volume might also increase lipophilicity, which is positively correlated with permeability (Garg et al. 2008).
5. The order of permeability appears to be active uptake compounds > passive diffusion compounds > efflux compounds (by about one log *PS* unit in each case), and the effect of molecular charge for log *PS* passive diffusion was basic compounds > neutral compounds > acidic compounds (Liu et al. 2004).
6. Much of the effort in QSAR studies has focussed on *finding and improving statistical* relationships between log BB and variables such as lipophilicity, PSA, molecular size, etc. However, given that the error in log BB (and log *PS*) experimental measurements is quite large, and a widely diverse range of compounds which have very different chemical structures, size, polarity, etc. have been examined, improvements in correlation coefficients may not necessarily be real (outliers may only be gross outliers). There are also significant errors in variables such as lipophilicity, polarity, size, etc. Multiple regression analysis is particularly error prone where log BB is correlated with 3–5 variables. There is a significant error in the log *PS* values themselves, since experimental conditions can vary amongst different studies. It is suggested that only a molecular mechanistic approach which starts from a sound physical–chemical basis on a structurally similar range of compounds can be meaningfully correlated with experimental permeability measures such as log *PS*. In silico methods based on QM, methods can help reduce errors in molecular properties and errors from wet chemical methods used in log *P* lipophilicity measurements.

Suenderhauf et al. 2012 recently used a decision tree analysis of 153 log *PS* data to find the dominant descriptors of permeability were lipophilicity (*a*Log *P*) and charge (PSA), with molecular geometry and connectivity also important factors. Their model also appeared to account for active transport as well as passive diffusion permeability. The property ranges used were molecular weight 46–1,201 Da, partition coefficient (*a*Log *P*) −4.3 to −2.4, polar surface area (PSA) 3.2–279 $Å^2$, rotatable bonds count 0–18 and hydrogen-bond acceptor count 1–23. A broad distinction was found between positive (CNSp+) and negative (CNSp−) molecules (compounds with log *PS* values ≥ -2 and ≤ -3, respectively, with log *PS* values between −2.1 and −2.9 were exempt from consideration). Huwyler's model is generally consistent with the previous regression QSAR models, but includes a broader range of physiochemical properties, and the analysis is not constrained to parameters that are used in QSAR regression models.

A significant issue relating to the many QSAR studies of BBB permeability is the distinction between passive diffusion and active transport processes. It is not clear how

many studies have made the distinction, since these two broad categories involve very different mechanisms. Recent work has shown that many of the higher molecular weight (volume) permeants utilise active transport mechanisms, such that true passive diffusion is not common (Pardridge 2007; Mangas-Sanjuan et al. 2010; Nau et al. 2010; Gabathuler 2010; Banks 2009; Pajouhesh and Lenz 2005; Jouyban and Soltani 2012; Pardridge 2012).

The characteristics of the BBB itself clearly are dominant in any mechanistic considerations of permeability by drugs or other compounds such as amino acids, etc. One important factor which affects the passage of highly polar and charged species is the *dipole potential* of the lipid bilayer membrane, (Stowasser 2008; Peterson et al. 2002; Cattelotte and Tournier 2009; Walter and Gutknecht 1986; Bezanilla 2008; Heimburg 2012; Koerner et al. 2011; Cafiso 1995) which has a phosphatidylcholine (PC) head attached to a long-chain fatty acid bilayer. Charged molecules can modify the membrane dipole potential by electrostatically interacting with the BBB membrane by attraction or repulsion. Positively charged molecules interact with the membrane, causing the N^+ end of the head group to move towards the water phase, away from the lipid membrane surface. Conversely, negatively charged molecules cause the N^+ end of the head group to move towards to the lipid surface. By changing the angle of the dipole with respect to the membrane surface, the membrane potential is altered. *Alteration of the membrane potential affects the permeability of charged ions through the BBB*. Exactly, how charged and polar species interact with the BBB is unknown, but using model lipid bilayer membranes such as dipalmitotylphosphatidylcholine (DPPC) or diphytanoylphosphatidylcholine (DPHYPC) the membrane potential has been measured at 243 ± 4 and 228 ± 5 mV, respectively (Cattelotte and Tournier 2009). However, while *formally* charged lipophilic molecules such as the tetraphenylborate anion and tetraphenylphosponium cation interact with PC lipid membranes (possibly because high charge dispersal to the phenyl rings allows the dominant molecular lipophilicity to facilitate passive diffusion through the membrane), uncharged species such as phloretin also interact with the PC lipid membranes. The suggested interaction between phloretin (which is known to lower the membrane potential) and PC lipid bilayers is a hydrogen bond between the phloretin and the P=O of the phospholipid, or the C=O of the lipid ester (Peterson et al. 2002; Cafiso 1995).

Charged species, including zwitterionic species such as amino acids such as glycine, alanine, etc., at the physiological pH 7.4 of blood serum can interact with the phospholipid membrane which can sense the charge on the interacting species (Bezanilla 2008) or by affecting the capacitance of the membrane and causing electromechanical changes in the membrane (Heimburg 2012), or other similar electrodynamic processes (Koerner et al. 2011). These electromechanical processes are related to the thermal fluctuation of defects or small mobile free volumes in the hydrocarbon phase of membranes which might allow passage of small molecules through the membranes (Trauble 1971). A molecular dynamics study (Chew et al. 2008) of three adamantanes with a model PC lipid bilayer membrane 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) has shown that the protonated species interact with the PS lipid head group, creating a deformation of the membrane. The positively charged ammonium group faces the negatively charged phosphate moiety, and remains in this orientation until the adamantanes reach the centre of the lipid bilayer, then flips to face the PC headgroup of the other lipid leaflets. By computing the pK_a as a function of lipid depth, it was concluded that *deprotonatio*n occurs, although it is unclear whether deprotonation occurred in the bulk solution of after initial adsorption into the interface region. This work will be shown to support a "preorganization" desolvation—dipole process prior to initial adsorption.

There are three separate potentials involved at the blood–membrane interface: (1) the trans-membrane potential, $\Delta\psi$, is the potential difference between the aqueous solutions on either side of the membrane. It arises from concentration differences of ions; (2) the surface potential, ψ_S, is the potential difference between the membrane surface and the aqueous bulk. It arises from fixed charges at the membrane/water interface, affecting the negatively charged head groups of lipid molecules; (3) the dipole potential, ψ_D, is the potential difference between the centre of the bilayer and the membrane/water interface. It follows that any relationship between a charged (or highly polar) species and the BBB membrane potentially involves all three types of potentials which importantly includes water molecules as well as the drug or other physiologically important molecules. Cafiso (1995) has suggested that a significant proportion of changes to the dipole potential of lipid membranes may involve electrodynamic alignment of the dipoles of bound water molecules, as well as the dipoles of charged drugs.

The importance of the membrane dipole potential for charged, zwitterionic, or highly polar neutral molecules (e.g. phloretin) in permeating the BBB membrane indicates an important feature of CNS drugs may be the dipole moment in water. Also it has been recently shown that the free energy of solvation is a dominant factor in deciding the ability of statins to cross the BBB (Fong 2014). The binding strengths of water molecules to the BBB membrane and the drug are expected to be crucial, if desolvation of charged or highly polar species are required before permeation can occur. Both passive diffusion and active transport processes through the membrane cells would be

affected, particularly if the aqueous hydration strengths are energetically significant. Active transport processes require carrier proteins, so desolvation would be required to facilitate protein–drug interaction.

Objects of This Study

1. To examine the molecular basis of BBB permeability focussing on the characteristics of molecules that can potentially permeate the BBB, and models of the BBB membrane itself such as DPPC, DPHYPC, dilauroyl phosphatidylcholine (DLPC), 1,2-dioleoyl phosphatidylserine (DOPSE), POPC, etc.
2. The permeability characteristics of molecules examined by quantum mechanical (QM) methods are the free energies of solvation, molecular volumes, atomic electrostatic charges, dipole moments, and measures of hydrogen-bond donor/acceptor and cavity effects in water. Both passive and active transport processes will be examined.
3. An examination of the membrane potential of the model PC lipid bilayer membranes DPPC, DPHYPC, DLPC and POPC will be undertaken using QM methods to probe how the membrane dipole potential affects the dipole and desolvation of molecules, and hence permeability through the BBB.
4. Log *PS* experimental data will be used as the kinetic measure of BBB permeability. While there have been many QSAR investigations of log BB with variables such as lipophilicity, PSA, hydrogen bonding, etc., log BB is an equilibrium measure which can be confounded with variables such as drug–blood protein interactions, metabolic disposal, etc. Log *PS* as a kinetic measure is less affected by these variables.

Experimental

All calculations were carried out using the Gaussian 09 package on optimised structures. Electrostatic potential at nuclei for solutions was calculated using the CHELPG method in Gaussian 09. The atomic charges produced by CHELPG are not strongly dependant on basis set selection. Using the B3LYP level of theory, calculated atomic charges were almost invariant amongst the basis sets 6-31 G(d), 6.311(d,p), 6-311+(2d,2p) and 6-311G++(3df,3dp) (Kubelka; Martin and Zipse 2005, Marenich et al. 2009). Errors between calculated and experimental dipole moments were 3 %. All solvent calculations were at the B3LYP/6-31G*(6d,7f) level of theory, using optimised geometries, as this level has been shown to give accurate electrostatic atomic charges, and was used to optimise the IEFPCM/SMD solvent model. Where a solvent study was carried out to compare different solvents, the same optimised solute geometry was used. With the 6-31G* basis set, the SMD model achieves mean unsigned errors of 0.6–1.0 kcal/mol in the solvation free energies of tested neutrals and mean unsigned errors of 4 kcal/mol on average for ions (Rayne and Forest 2010). It has been found that the B3LYP/6.31G+* combination gives reasonably accurate PCM and SMD solvation energies for some highly polar polyfunctional molecules, which are not further improved using higher level basis sets (Carpenter et al. 2014). Adding diffuse functions to the 6-31G* basis set (i.e. 6-31^{+}*) had no significant effect on the solvation energies with a difference of ca 1 % observed, which is within the literature error range for the IEFPCM/SMD solvent model.

It should be noted that some very low correlation coefficients R^2 are shown in some of the regression equations. These low values are associated with very low slopes, i.e. the relationships with log *PS* are quite insensitive, which is a known statistical issue with regression correlations. The standard error of the estimate (log *PS*), SEE, is a better indicator of the precision of regression equations. In most cases examined, a lack of sufficient log *PS* observed data makes the multiple regression equations less robust than desirable.

Compounds analysed by Liu et al. (2004) are as follows: Antipyrine, Caffeine, Threophylline, Threobromine, CP-141938*, Fluoxetine, Chloroambucil, Colchicine*, DPDPE*, Daunomycin*, Digoxin*, Dopamine, Glycine, Hypoxanthine, Xanthine, Levodopa*, Methotrexate, Morphine*, NFPS, Phenylalanine*, Phenytoin, SR141716, Quinidine*, Salicyclic Acid, Taurocholic Acid, Valproic Acid and Testosterone. Nine compounds* were identified as being actively transported across the BBB.

Results and Discussion

It is clear from the many reviews of BBB permeability that drug and physiologically important compounds are primarily dependant on lipophilicity, polarity or charge, hydrogen bonding and molecular size. In this study, these measures are calculated by QM solvent effects using the solvation free energy $\Delta G_{\text{octanol}}$ for *n*-octanol as a measure of lipophilicity, the dipole moments or the calculated atomic charges (CHELPG charges) in water as measures of polarity or molecular charge, and the calculated molecular volumes. This approach has been previously applied to statins. (Fong 2014) Unfortunately, even though log *P* or log *D* in water–*n*-octanol (or other partitioning solvent combinations) is widely used to define drug lipophilicity, *n*-octanol contains 2.18 M water in partitioning experiments at equilibrium, so most polar solutes would be solvated by this water, indicating that the log *P* or log *D* values may be suspect. Calculated *C*log *P* values are based on experimental log *P* values. There

Table 1 Selected log *PS* and input data used to derive Eqs. 1–9 and to analyse closely related test compounds

	Log *PS*	ΔG_{water}	$-\Delta G_{octanol}$	$V_{octanol}$	D_{water}	ΔG_{CDS}
Water	−2.1	8	−6.7	12	2.4	1.5
Methanol	−1.6	3.6	−4.2	30.5	2.2	2.5
Ethanol	−1.5	4.8	−5.1	53	2.2	2.4
2-Propanol	−1.7	4.5	−5.5	56.9	2.2	2.7
Ethylene glycol	−3	10.1	−8.8	54.7	3	2.5
Propylene glycol	−2.5	8.8	−8.5	63.3	3.1	2.9
1,4-Butanediol	−3	10.9	−10.7	77.4	3.1	3
1-Butanol	−2.4	4.5	−6.2	71.5	2.2	2.9
Xanthine	−3.8	18.3	−14.6	85	4.8	2.4
Caffeine	−2	15.2	−12	147.3	4.3	0
Threophylline	−2.9	16.5	−13.2	112.7	4	0.8
Threobromine	−3	15.1	−11.9	126.9	4.4	0.9
Hypoxanthine	−3.5	19.1	−16.8	102	6.3	1.8
Morphine	−2.7	17.7	−17.2	4.7	233.4	2.5
Morphine ion		70.1	−66.7	17.8	204	6.4
Heroin	−1.2	8.2	−17.8	4.9	286.6	5.9
Heroin ion		69.7	−66.6	20	256.4	9.7
Codeine	−1.7	16.8	−16.5	4.6	204.2	3.5
Codeine ion		68.2	−65	18.9	209.2	7.3
Morphine-6-glucuronide	−3	42.2	−35.9	4.8	292.3	5.8
Morphine-6-Glucur Zwitt		1,119.1	−103.5	42.1	337.7	9.6
Methadone	−1.3	6.1	−12.6	6.7	287.8	8.9
Methadone ion		52.4	−54.5	12	248.7	8.9
Antipyrine	−2.0	11.4	−12.6	173.1	8.1	5.0
Iodoantipyrine	−1.1	12.3	−12.2	140.1	8.4	4.6
Urea	−3.8	13.6	−11	37.5	5.7	3.3
Thiourea	−3.4	11.4	−10.7	31.6	5.9	1.6
Nicotine	−1.0	6.0	−7.7	136.3	3.0	1.4
Nicotine ion	Negligible	63.5	−61.0	120.8	10.7	3.9
N-Me nicotine ion pyrrole	Negligible	65.9	−59.9	111.1	9.6	2.3
N-Me nicotine ion pyridine	Negligible	51.8	−61.0	120.8	9.5	3.5

Log *PS* values from (Goodwin and Clark 2005; Abraham et al. 1997; Liu et al. 2004; Suenderhauf et al. 2012) and reference therein

ΔG values in kcal/mol, molecular volume V values in cm^3/mol, dipole moment values in D

ΔG_{CDS} in kcal/mol, include hydrogen-bonding interactions, solute–solvent cavity interactions and other non-electrostatic solute–solvent effects

Log *PS* data for nicotine and derivatives from (Oldendorf et al. 1979, 1993)

are also significant errors in obtaining experimental log *P* values. It is also clear that *n*-octanol has significant hydrogen-bonding capability, whereas *n*-octane has none. However, *n*-octanol has been widely accepted as a membrane bilayer-mimicking solvent, where the 2.18 M equilibrium water concentration is consistent with the known water levels in cell membrane bilayer cores because of trans-bilayer transport (Balaz 2009).

It has been previously shown (Fong 2014) that desolvation effects (as measured by ΔG) can be dominant in BBB permeability of statins. There have been previous QSAR studies of the linear relationship between the free energy of solvation and log BB (Lombardo et al. 1996; Keseru and Molnar 2001) which have suggested that compounds with log BB > 0.3 cross the BBB, while those with log BB < -1.0 do not. Importantly, from a screening survey of 8700 CNS drugs, it was found that 96 % of CNS active drugs had a ΔG higher than -12 kcal/mol. This study not only examines the desolvation of a wide range of drugs and their log *PS* permeability, but also concomitantly examines variables such as dipole moment or atomic charge, molecular volume, hydrogen bonding and solvent cavity effects at the same time.

To gain mechanistic insight into the molecular basis of passive diffusion-based permeability through the BBB, a closely related series of alcohols has been examined. Unfortunately, the QSAR approach of using a widely diverse range of compounds does not lend itself to mechanistic interpretation by linear free energy analysis (only statistical inference), as there is no control of molecular variability or transport processes. These results are shown in Table 1. It is clear that there are strong relationships between log *PS* and ΔG_{water} (positive), a strong positive relationship with membrane lipophilicity as measured by $\Delta G_{octanol}$, a negative relationship with dipole moment D_{water} and a weaker negative relationship with molecular volume. It is clear that the value for water (and

ethylene glycol to a lesser extent) is a clear outlier for the ΔG_{water} and volume relationships, which is consistent with the known anomalous properties of this unique highly polar solvent. All molecules were geometry optimised to give the lowest energy conformations. There is a weaker negative relationship between log *PS* and CDS in water. The CDS term is included in the overall ΔG_{water} term, and is a measure of hydrogen bonding (based on Abraham's coefficients) and cavity effects (creation of a "hole" in the solvent in which to place the solute, plus other cavity interaction effects).

The data are consistent with the following model for BBB permeability:

1. *Desolvation*, the reverse of solvation, of the permeating drug is a dominant negative factor, in view of the large ΔG values, and is probably the rate determining step (RDS).

$$\text{Log } PS = -0.54\Delta G_{water} - 2.96 \text{ with } R^2\ 0.79,\ \text{SEE } 0.98, \quad (1)$$

where ΔG_{water} is the water desolvation free energy

2. *Lipophilicity* as measured by $\Delta G_{octanol}$ is significant and highly positively correlated with the permeation through the lipid bilayer. As *n*-octanol is a proxy for the membrane bilayer, this relationship implies that once desolvation of water has occurred, and the alcohols start to permeate the lipid bilayer, lipophilicity determines the rate of diffusion.

$$\text{Log } PS = 1.28\Delta G_{octanol} - 2.83 \text{ with } R^2\ 0.80,\ \text{SEE } 1.24, \quad (2)$$

where $\Delta G_{octanol}$ is the solvation free energy in *n*-octanol or lipophilicity.

3. The *dipole moment D* in water is negatively correlated, probably due to the effect of the membrane dipole potential as the drug approaches the cell wall. This effect is much smaller than desolvation or lipophilicity.

$$\text{Log } PS = -1.40\,D + 2.25 \text{ with } R^2\ 0.71,\ \text{SEE } 1.04, \quad (3)$$

where *D* is the dipole moment in water.

4. *Molecular volume V* is negatively correlated to permeability, probably due to how well the drug can physically (sterically) enter the lipid bilayer and diffuse through.

$$\text{Log } PS = -0.82V - 41.96 \text{ with } R^2\ 0.41,\ \text{SEE } 1.62, \quad (4)$$

where *V* is the molecular volume in *n*-octanol (water excluded as an outlier).

It should be noted that the alcohols studied here are relatively small in size, which might be expected to favour passive diffusion.The multiple regression model Eq. (5) for BBB permeation is

$$\text{Log } PS \sim 0.20\,\Delta G_{water} + 1.03\Delta G_{octanol} - 0.044D + 0.006V, \quad (5)$$

where R^2 0.855, SEE 1.04, F 4.43.

This relationship is only indicative, since there are insufficient data points to be statistically robust. *The linear relationships in 1 to 4 above are more statistically meaningful.*

The model is consistent with previous QSAR models, but puts together the four factors for the first time, particularly using the free energy of desolvation and the dipole moment which have not been previously considered. Hydrogen-bonding properties are captured in the solvation/desolvation terms. A dependency on lipophilicity and molecular size is consistent with previous QSAR models. This approach is unique in using a full in silico QM approach.

It should be noted that this model applies to BBB diffusion processes, where the molecular volumes are relatively small (12–77.4 cm^3/mol), and all compounds are neutral species. If active transport processes are involved (Wu et al. 1997), desolvation must still occur prior to any carrier protein–drug interaction, based on the magnitude of ΔG_{water}. It is unlikely on energy grounds that a *large* charged molecule could permeate a cell membrane in a manner that smaller ions (e.g. Na^+) can enter ATP-driven ion pores. Lipophilicity and molecular volume are also likely factors, based again on energy considerations and previous QSAR results (Suenderhauf et al. 2012; Hutt 2006; Wu et al. 1997). The dipole moment of the drug is a vector measure of the molecular charge separation, and since protein–drug interaction is essentially an electrostatic interaction, it seems intuitively likely that the dipole of the drug should be an important factor in any drug–protein carrier interaction. Charged and zwitterionic molecules will be influenced by the membrane dipole potential which has a negatively charged PC head group with a positively charged lipid bilayer tail (see Sect. 7 below). So the membrane dipole potential can exert a force on larger charged molecules that might facilitate desolvation processes prior to passive or active transport into the cell membrane, as shown previously (McCall et al. 1982).

Active transport processes involve a protein–drug interaction where a neutral drug species can electrostatically interact with the carrier protein. Desolvation of water from the drug, which may be in a charged or zwitterionic state in blood plasma at pH 7.4, has to first occur, which is energy expending. Passive trans-cellular or paracellular diffusion is also favoured by lower charge which increases lipophilicity, so desolvation of charged or zwitterionic species to give a *neutral* species in a lipophilic environment favours passive permeation. By examining the effect of pH on the (active) permeability of D-glucose, L- and D-lactate, and

nicotine (passive), it was found that it is the uncharged species that exhibits much higher permeation rates for both active transport and passive transport (Oldendorf et al. 1993).

To test this model, some comparisons have been made with *closely related compounds or series of compounds* which are known to show significant differences in permeability. These tests include:

1. Xanthines: Xanthine, caffeine, theophylline and theobromine are very closely related drugs structurally differing only in methyl groups substituting hot H atoms, and hypoxanthine is also closely related, but having only one carbonyl group instead of two as in the other xanthines. An analysis shows the flowing linear relationships exist:

$$\text{Log } PS = -0.30\,\Delta G_{\text{water}} + 2.08 \text{ with } R^2\ 0.64,\ \text{SEE } 0.48, \quad (6)$$

where ΔG_{water} is the water desolvation free energy

$$\text{Log } PS = 0.24\Delta G_{\text{octanol}} + 0.24 \text{ with } R^2\ 0.64,\ \text{SEE } 0.55, \quad (7)$$

where $\Delta G_{\text{octanol}}$ is the solvation free energy in *n*-octanol or lipophilicity

$$\text{Log } PS = -0.41D - 1.11 \text{ with } R^2\ 0.30,\ \text{SEE } 0.67, \quad (8)$$

where D is the dipole moment in water (the D of hypoxanthine is an outlier, but is still included in the analysis)

$$\text{Log } PS = 0.03V - 6.22 \text{ with } R^2 = 0.915,\ \text{SEE } 0.23, \quad (9)$$

where V is the molecular volume in *n*-octanol. These Eqs. 6–9, using only 5 log *PS* data points and consequently of low statistical robustness, are similar to those equations using the alcohol data (Eqs. 1–4). The main difference is that the relationship with molecular volume (which range from 85 to 147 cm^3/mol) shows an inverse relationship from that of the much smaller alcohols (which range from 12 to 77.4 cm^3/mol). This is consistent with the observation that the relationship between permeability and molecular size has a parabolic relationship (Garg et al. 2008).

Despite the less than rigorous statistical basis (because of insufficient log *PS* data), the *model performs reasonably well in closely related series of compounds being transported by facilitated and passive diffusion processes*. Caffeine, theophylline and hypoxanthine are known to be transported by both active and passive modes (Habgood et al. 1998; Spector 1987; McAllister 2001).

2. Morphine and related derivatives: Morphine, heroin, codeine and morphine-6-glucuronide (M6G) are all related: heroin has the two hydroxyls of morphine replaced by acetyl groups, but shows a 31-fold to 100-fold penetration rate of the BBB compared to morphine. M6G has a glucoronide group substituted for the 6 hydroxyl group of morphine and has a decreased BBB penetration rate of 57 times compared to morphine under *identical* conditions (Oldendorf et al. 1972). M6G is the major active metabolite of morphine, and heroin, and is responsible for much of the analgesic effect. Codeine which has the phenol hydroxyl group of morphine replaced by a methoxy group penetrates the BBB ten times faster than morphine. Morphine is actively transported across the BBB by P-glycoprotein (Liu et al. 2004). Codeine crosses the BBB by passive paracellular diffusion (McCaffrey and Davis 2012). The log *PS* values of morphine, heroin, codeine and M6G are −2.7, −1.2, −1.7 and −4.5, respectively. These compounds all exist predominantly as the cations or as a zwitterion (M6G) at physiological pH levels.

Examination of the ΔG_{water}, $\Delta G_{\text{octanol}}$, D and V values reveals that the zwitterionic M6G has larger desolvation ΔG_{water} value (49.0 kcal/mol greater) and lower lipophilicity for the neutral species as measured by $-\Delta G_{\text{octanol}}$ (18.7 kcal/mol) but larger D (by 2.36 times) and larger V (by 1.65 times) which appears to explain why it permeates 57 times as slow as the morphine ion under identical conditions. M6G is also 187 times less lipid soluble than morphine as measured by octanol/water partitioning (Oldendorf 1972).

Examination of the difference between morphine ion and heroin ion does not reveal such large differences as seen for M6G. Morphine is more soluble in water, and has a smaller desolvation energy 0.4 kcal/mol, a higher neutral lipophilicity by 0.6 kcal/mol, a lower D by 12.4 % and a smaller V by 33 % than the heroin ion. All these factors suggest that morphine should permeate faster than heroin. However, heroin is *more soluble* than morphine in lipids because of the two acetyl groups (McCaffrey and Davis 2012). These data suggest the 30.6 (to 100) times faster permeation rate for heroin (Liu et al. 2004; Jenkins 2008) is dominated by the heroin-lipophilic protein solubility.

Comparison of the morphine ion with the codeine ion shows that codeine has a smaller desolvation energy by 1.9 kcal/mol, a higher neutral lipophilicity by 0.7 kcal/mol, a higher D by 6.1 % and a larger V by 2.5 %, which is consistent with the observed difference in log *PS*.

In summary, morphine, heroin, codeine and M6G which are transported across the BBB by active and

passive processes appear to be consistent with the developed transport model when solubility is taken into account.

3. Antipyrine and iodoantipyrine have experimental log *PS* values of −2.0 and −1.1, respectively, although the difference is only an I atom substituting for a H atom. The ΔG_{water} for antipyrine is lower by 0.9 kcal/mol (therefore, requiring a lower desolvation energy, which is a positive factor for permeation), $\Delta G_{octanol}$ is lower by 0.4 kcal/mol (therefore, less lipophilic which is a negative factor for permeation), the dipole moment in water is lower by 0.3D (which is a positive factor for permeation), and the molecular volume is larger by 19 % in *n*-octanol (which is a negative factor for permeation) compared to iodoantipyrine. Apparently, the greater molecular volume in octanol and increased lipophilicity override the desolvation and dipole effects to make iodopyrine permeates faster.
4. Urea and thiourea have experimental log *PS* values of −3.8 and −3.4, respectively, although the difference is only an S atom substituting for a O atom. The ΔG_{water} for urea is higher by 2.2 kcal/mol, $\Delta G_{octanol}$ is lower by 0.3 kcal/mol, the dipole moment in water is lower by 0.2 *D*, and the molecular volume is larger by 16 % in *n*-octanol compared to thiourea. The large difference in ΔG_{water} is due to a greater ΔG_{CDS} for urea, probably due to a greater hydrogen-bonding interaction. It appears that the larger desolvation for urea is the main cause of its lower permeation rate.
5. Nicotine (log *PS* −1.0) in its protonated form shows a greatly decreased brain uptake index (BUI) from 109 (pH 7.2) to 49 (pH 4.7) (Oldendorf et al. 1993). The difference in ΔG_{water} for nicotine and the protonated species is 57.5 kcal/mol, $\Delta G_{octanol}$ is 53.4 kcal/mol, the dipole moment in water differs by 7.7D, though the molecular volume of nicotine is larger by 12.8 % in *n*-octanol. Similarly, the two *N*-methyl salts of nicotine (quaternized at either the pyridine or pyrrole N atoms) showed BUI values in rats of 3 compared to nicotine 120 at pH 7.4. The differences in ΔG_{water} for nicotine and the *N*-methyl species are 59.9 or 45.8 kcal/mol, $\Delta G_{octanol}$ are 52.2 or 43.6 kcal/mol, the dipole moments in water differs by 6.6 or 6.5 D, and the molecular volumes of nicotine are smaller by 18.5 % (pyrrole *N*-methyl) or larger by 6.6 % (pyridine *N*-methyl) in *n*-octanol, respectively. Overall, these data are consistent with the higher passive diffusion uptake index for the neutral nicotine species, clearly demonstrating that large desolvation, lipophilicity and dipole factors are operating.
6. Stereoselectivity of transport across the BBB: there are many examples of the chirality of drugs affecting their pharmacology (Hutt 2006), ranging from enantiomeric differences in binding to plasma proteins to transport across the BBB. As these enantiomers have almost identical physical and chemical properties, they represent a good test of any theory being developed for BBB permeability. It is generally thought that the origins of stereoselectivity are electrostatic in origin, possibly during protein binding which involves conformational selectivity in the active transport process (Hutt 2006). Several examples of stereoselective permeation have been investigated:

   I. 4-Fluoro-L-phenylalanine has a log *PS* −1.7 compared to 4-fluoro-D-phenylalanine log *PS* −2.9: The ΔG_{water} for 4-fluoro-L-phenylalanine (zwitterion at pH 7.4) is higher by 0.6 kcal/mol, $\Delta G_{octanol}$ neutral species is lower by 1.9 kcal/mol, the dipole moment in water is almost the same, and the molecular volume is smaller by 6.4 % in *n*-octanol than the 4-fluoro-D-phenylalanine zwitterion. These data do not appear consistent with the log *PS* data, unless the molecular volume term dominates. The $\Delta G_{octanol}$ value has been used as a proxy for lipophilicity of the membrane bilayer in passive diffusion permeation. However, for active transport (by the large amino acid transporter, LAT1 (Wu et al. 1997) where a drug–carrier protein interaction is involved, it is unclear whether the carrier protein–drug interaction is hydrophobic driven, or hydrophilic driven where hydrogen bonding dominates. The experimental result could be explicable if the positive desolvation and smaller molecular volume are supported by a postive contribution to the permeability rate by a lower $\Delta G_{octanol}$, implying that the protein–drug interaction has a dominant hydrophilic rate determining effect.
   II. D & L amino acids: The transport of amino acids across the BBB is by active transport processes (Oldendorf and Szabo 1976; Hawkins et al. 2006; Smith 2000; Torres and Raul 2003). Using the brain uptake index (BUI) as a rate measure of BBB permeability, it was found that the L-enantiomers of aspartic and glutamic showed higher BUI than the D-enantiomers. The ΔG_{water} for L-aspartic acid (anion) is higher by 1.1 kcal/mol, $\Delta G_{octanol}$ for the neutral species is lower by 2.4 kcal/mol, the dipole moment in water is identical, and the molecular volume is virtually the same in *n*-octanol than the D-aspartic acid anion. The

ΔG_{water} for L-glutamic acid (anion) is lower by 0.3 kcal/mol, $\Delta G_{octanol}$ (neutral species) is the same, the dipole moment in water is higher by 1.3D, and the molecular volume is smaller by 18 % in *n*-octanol than the D-glutamic acid anion. As an organic anion transporter is the active transporter, these data appear inconsistent with the simple model, and show the same pattern as that for 4-fluoro-phenylalanine. It appears that active transport of neutral amino acids by LAT1 is clearly more complex than for passive diffusion transport.

III. Lactic acid: the L-enantiomer of lactic acid showed higher BUI than the D-enantiomers (Oldendorf and Szabo 1976). Lactic acid is predominantly transported across the BBB by the monocarboxylic acid transporter type 1, MCT1 (Wu et al. 1997). The ΔG_{water} for L-lactic acid (anion) is higher by 3.2 kcal/mol, $\Delta G_{octanol}$ (neutral species) is higher by 1.0 kcal/mol, the dipole moment in water is lower by 2.8 D, and the molecular volume is smaller by 3 % in *n*-octanol than the D-lactic acid anion. These data are consistent with the model, and the smaller molecular size is consistent with *facilitated* diffusion transport. It has been previously shown that the neutral species that penetrates the BBB fastest (Oldendorf et al. 1993) which is consistent with desolvation being an important "preorganization" factor for facilitated transport, before permeation initiates.

IV. Glucose: D-glucose is transported across the BBB by the GLUT-1 transporter about 100 times faster than its stereoisomer L-glucose (log *PS* 2.5 vs. 5.0, respectively). The only differences between the stereoisomers are a larger molecular volume in water and octanol by 34.4 and 8.6 % for the open-chain forms of D- and L-glucose. This situation is reversed for the D- and L-pyranose form. This apparent anomaly must be due to a stereospecific glucose–GLUT-1 interaction as it is not explainable on the basis of any "preorganization" processes related to changes from the bulk solvent (blood serum) or prior interaction between glucose–BBB before permeation of the BBB start to occur (e.g. desolvation, lipophilic solubility or dipole). Glucose transport at the BBB appears to be dependent on and regulated by a serial chain of membrane-bound and intracellular transporters and enzymes (permeases that change their conformations during the transport processes) (Oldendorf et al. 1979).

V. Baclofen: R-Baclofen (a CNS muscle relaxant) was shown to have a rat BBB transport rate 4.3 times as fast as the S-isomer, probably using the large neutral amino acid carrier, since it is a zwitterion at pH 7.4 and has low lipophilicity (van Bree et al. 1991). The ΔG_{water} for R-baclofen (zwitterion) is higher by 0.7 kcal/mol, $\Delta G_{octanol}$ neutral is higher by 2.5 kcal/mol, the dipole moment in water is higher by 3.5 D, and the molecular volume is higher by 4 % in *n*-octanol than the S-baclofen zwitterion. As active transport (large neutral amino acid transporter) is involved, these data are difficult to interpret unambiguously using the passive transport model.

VI. Mefloquine: Mefloquine is a chiral neurotoxic antimalarial agent showing stereoselective brain uptake in humans and rats. It is a substrate and an inhibitor of the efflux protein P-glycoprotein. (−) Mefloquine had a lower blood and brain apparent volume of distribution and a lower efflux clearance from the brain, resulting in a larger brain–blood ratio compared to (+) mefloquine (Ding 2004). The ΔG_{water} for (−) mefloquine (cation at pH 7.4) is higher by 2.2 kcal/mol, $\Delta G_{octanol}$ neutral is higher by 1.0 kcal/mol, the dipole moment in water is lower by 0.5 D, and the molecular volume is smaller by 13 % in *n*-octanol than the (+)-mefloquine cation. The data are ambiguous as the higher desolvation energy and smaller volume favour the faster permeation by the (+) stereoisomer, but the $\Delta G_{octanol}$ and D support a faster rate for the (−) stereoisomer (Barraud de Largerie et al. 2004).

VII. Ritalin: Ritalin is widely prescribed for attention-deficit hyperactivity disorder (ADHD). The *d*-threo isomer is the pharmacologically active species, but the l-threo isomer crosses the BBB faster by a factor of about 2:1 to 5:1 (Spector 1988). The ΔG_{water} for l-threo isomer (cation at pH 7.4) is lower by 1.0 kcal/mol, $\Delta G_{octanol}$ (neutral) is lower by 1.0 kcal/mol, the dipole moment in water is higher by 0.4 D, and the molecular volume is larger by 15 % in *n*-octanol than the *d*-threo isomer cation. As the active monoamine transporter is involved (Hawkins et al. 2006; Smith 2000; Torres and Raul 2003), the data are ambiguous in terms of which stereoisomer should permeate faster.

VIII. Quinine (an antipyretic, anti-inflammatory and antimalarial) log *PS* −2.6 and quinidine (an antirrhythmic) log *PS* −3 are stereoisomers.

The ΔG_{water} for quinine (cation at pH 7.4) is lower by 0.2 kcal/mol, $\Delta G_{octanol}$ neutral is almost identical, the dipole moment in water is lower by 0.3 D, and the molecular volume is larger by 3 % in *n*-octanol than the quinidine cation. Active transport is probably involved in view of the large molecular sizes.

IX. It is apparent from the experimental data above (6, I–VIII) that there are relatively small differences in the desolvation ΔG_{water}, lipophilicity $\Delta G_{octanol}$, dipole moment in water, and molecular volume in *n*-octanol for all the stereoisomers examined. *There is no consistent pattern since all these examples involve active transport processes, where there are differences in the trans-membrane proteins and their interactions with different drugs*. In the case of glucose, it is clear that a specific stereoselective active transport process prevails, which is not dependent at all on any "pre-organisation" of the permeant prior to commencement of permeation into the BBB. The test data where *facilitated diffusion* is involved for smaller sized permeants (xanthines, ureas, antipyrines and lactic acids) appear to support the four factor passive diffusion model Eqs. (1–5).

7. The permeability model developed above assumes that *n-octanol is a proxy for the lipid bilayer membrane of the BBB*, and is a measure of lipophilicity of the BBB. For charged or zwitterionic species, it has been assumed that it is the *neutral species* that passively permeates or is actively transported by a carrier protein, and so $\Delta G_{octanol}$ is calculated for the neutral species. Also it has been shown above that the dipole moment of the drug is one of the four critical factors in the permeation model for the BBB. To test the assumption about *n*-octanol as a valid proxy for lipophilicity in the BBB, and to explore physical mechanisms that explain why the dipole moment should be a critical factor in permeation, the model lipid bilayer membranes, DPPC, diphytanoylPC (DPHYPC), dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), DOPSE and POPC and their interactions with various compounds have been examined.

I. DPHYPC was constrained to a structure whereby the two parmitoyl ester chains are as close to parallel as possible (after molecular mechanics optimisation) and pointing away from the phosphatidylcholine (PC) head group, to resemble as closely as possible the PC lipid bilayer of a cell membrane. The relationship between ΔG for the DPHYPC and *n*-octanol for the range of alcohol solvents used to develop the permeation model, plus other alcohols up to 1-decanol, shows that a strong linear relationship is observed:

$$\Delta G_{DPHYPC} = 9.34\Delta G_{Octanol} - 6.98\left(R^2 0.976,\ \text{SEE } 0.75\right). \quad (10)$$

Water, and to a lesser extent, ethylene glycol, are outliers, presumably related to multiple hydrogen-bonding effects. This result indicates that *n*-octanol is a good proxy for a cell membrane bilayer.

II. The electrical properties of the BBB have been examined by calculating the charge distribution of the model membranes in water, all of which have a zwitterionic phosphatidylcholine PC head group and long bilayer fatty acid tails. It is known that DOPSE which has a negatively charged PC head group moves against the direction of an applied electric field (Koerner et al. 2011). Conversely, the model zwitterionic lipid bilayer membrane DLPC in the zwitterionic buffer 3-(*N*-morpholino)propanesulfonic acid (MOPS) at pH 7 becomes positively charged in an applied electric field. The addition of the ion Br^- to DLPC vesicles resulted in a negative charge on DLPC. Using X-ray techniques, it was found that the buffer solutes (MOPS, etc.) enter the inter-lamellar space and modify (possibly by binding) interlayer interactions (Koerner et al. 2011). These experimental results are consistent with electrostatic atomic charge models of DOPSE, DLPC and DLPC–MOPS which clearly show that the PC head group is overall negatively charged, and the bilayer lipid tail is slightly positively charged. For example, DOPSE has a overall −1.115 V PC charge, and a 0.04 V lipid tail. The DOPSE PC group has a charge of −3.69 V on the zwitterionic N and PO_4 atoms. (Similarly, the negatively charged DLPC–Br where the Br^- ion interacts with the P atom at a distance of 3.3 Å has charge of −3.60 V on the zwitterionic N and PO_4 atoms.) It is likely that charge in an electric field would be determined by this charge. DLPC–MOPS interacting through a weak electrostatic bond 2.6 Å between the P atom of DLPC and the O of the SO_3 group of MOPS has an overall −1.38 V PC charge and 0.35 V lipid tail. The DLPC–MOPS PC group has a charge of −1.89 V on the zwitterionic N and PO_4 atoms. It appears that the difference of −1.8 V between

the DOPSE and DLPC–MOPS zwitterion charges is instrumental in deciding the overall movement in an electrical field. Movement in an electrical field would be a complex interplay of charge-driven ionic factors for all cationic and anionic species present in solution. The dipole moments in water of DOPSE, DLPC–Br and DLPC–MOPS are 38.5, 31.0 and 37.1 D, respectively. These data all suggest that the BBB membrane would electrically interact with charged species and hence affect trans-membrane transport.

III. It has been shown that organic ions such as tetraphenylborate TPB^- and tetraphenylphosphonium TPP^+ can adsorb to and permeate lipid membranes owing to their hydrophobic nature and the strong delocalisation of charge to the phenyl rings (Cafiso 1995). TPB^- permeates about 10^6 times faster than TPP^+ and the difference in the free energy of binding to the membrane is about −5 kcal/mol. Hydrophobic anions generally bind more strongly than hydrophobic cations, and permeate through membranes faster (Stowasser 2008; Cattelotte and Tournier 2009; Cafiso 1995). The positive dipole potential of the mouse BBB restricts the permeability of cationic compounds by active transport mechanisms, but *neutral* compounds like phloretin or anionic species like TPB^- can enhance the permeability of cationic species (Cattelotte and Tournier 2009). DPHYPC-TPB^- (where the B weakly interacts with the P at 4.3 Å) has been compared to DPHYPC-TPP^+ (where the P of TPP^+ weakly interacts with the O of the PO_4 group at 2.6 Å): the dipole moments are 39.9 and 50.4 D, respectively, and ΔG_{water} are −117.4 and −115.9 kcal/mol, respectively. The DPHYPC-TPB^- PC group has a charge of −4.67 V on the zwitterionic N and PO_4 atoms, compared to DPHYPC–TPP^+ −5.1 V. These data support the experimental greater permeation rate and of TPB^- since the charge separation (as per the *D* values) is far greater for the DPHYPC–TPP^+, as well as the lower salvation energy and N and PO_4 charges of the DPHYPC–TPB^-. These data support the importance of a electrostatic relationship affecting the interaction between drugs and other permeants with the membrane potential, and consequently BBB permeability. Certain polar neutral compounds, like phloretin, and ionic compounds that have a high lipophilicity (and highly dispersed formal charge) can facilitate and permeate PC lipid membranes, so it is likely that similar relationships may apply to the BBB. An important aspect of how the membrane potential can interact with permeants is the concept of *voltage sensing* (Bezanilla 2008. Charged molecules can reorient in the electric field of the membrane (particularly at the negatively charged PC head of the membrane), as has been found in voltage-gated ion channels. Such a process might facilitate desolvation of permeants, as previously discussed above (Fong 2014; McCall et al. 1982). *This process is particularly important for active transport processes.*

Carpenter et al. (Liu 2005) have shown from molecular dynamics studies that an initial stabilising interaction of up to 3 kcal/mol occurs as the drug moves from the bulk solvent to the PC headgroup in the model membrane DOPC (about 2.5–3.0 nm from the bilayer centre). The most stabilising region is about 1.0–1.5 nm from the bilayer centre, which reflects the hydrophobic stabilisation as the drug penetrates the bilayer. Solvation energies were not explicitly investigated.

IV. Adamantidine has been used in the treatment of Parkinson's disease and influenza. It crosses the BBB log *PS* −3.1. Molecular dynamics modelling of the interaction of adamantidine with the model membrane POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine, has shown that adamanatidine firstly interacts with the negatively charged PC head group via the charged ammonium group, and is deprotonated as it penetrates the centre of the lipid bilayer (McCall et al. 1982). This work has focussed on the desolvation of charged or zwitterionic species in blood serum at pH 7.4 *before* permeants enter the cell membrane, so the neutral species is the permeating species. This is based on energy considerations since the desolvation of charged or zwitterionic species is highly energy intensive. The interaction of the dipole moment of permeants is also critical to the process of desolvation and hence permeation. A QM study of the adamantidine–POPC interaction shows

a. The difference in desolvation energy between the protonated species and neutral species is 60 kcal/mol in water.
b. The zwitterionic species in water can interact with the phosphate group via an

ionic NH_3^+–$^-$OP(O)– interaction or with the carbonyl group of the ester fatty acid via a NH_3^+–O=C– hydrogen-bonding interaction. The dipole moment of the {adamantidine-POPC} complex in water increases from 28.9 to 49.4 D, (compared to the value for uncomplexed POPC of 27.3 D). This dramatic change indicates that the dipole of POPC (and all the PC membrane models studied here), which is oriented from the negatively charged PC head group towards the positively charged lipid bilayer, has greatly increased negatively charged PC head group upon complexing with the adamantidine zwitterion. Such a large energy change could electromechanically distort the membrane to facilitate permeation (Bezanilla 2008; Heimburg 2012; Koerner et al. 2011; Cafiso 1995; Trauble 1971; McCall et al. 1982), as well as facilitate desolvation, since shedding hydrogen-bonded water molecules is a means of lowering the energy and stabilising the complex.

c. Permeation of the neutral adamantidine species into the lipid bilayer was studied by inserting the adamantidine molecule between the two lipid chains in two orientations, one with the $–NH_2$ moiety facing towards the PC head group, and the other with the $–NH_2$ group facing away from the PC head group. The dipole moments for the orientations are 23.9 and 30.4 *D*, respectively, in octanol, (or 20.7 and 24.9 *D* no solvent). This large difference of 6.5 *D* clearly illustrates the energy gradient as adamantidine permeates the lipid bilayer of POPC and then flips orientation and starts to interact with the charged head group of the other lipid leaflet that comprises the cell membrane. This finding is an accord with the previous MD study of POPC-adamantidine permeation (McCall et al. 1982). The magnitude of the change in dipole is large illustrating substantial energy changes to the lipid bilayer structure during permeation, presumably by electromechanical forces (Stowasser 2008; Peterson et al. 2002; Cattelotte and Tournier 2009; Walter and Gutknecht 1986; Bezanilla 2008; Heimburg 2012; Koerner et al. 2011; Cafiso 1995; Trauble 1971; McCall et al. 1982).

d. Adamantidine and other positively charged species can interact with either or both of the phosphate group of the PC head group or the carbonyl group of the lipid ester chain. It is also possible that interaction with the carbonyl could be via the neutral species, as the carbonyl group is directly attached to the lipid chains, so close to being in a hydrophobic environment. The dipole moments for the neutral species are 27.2 and 27.3 *D* in water and octanol, and for the charged species are 28.9 and 28.3 *D*, respectively. These small differences suggest that the neutral species preferentially interacts with the carbonyl group. However, the change in membrane potential by interaction with the carbonyl is clearly far smaller than the interaction with the phosphate group, so significant electromechanical changes in the membrane are probably not induced by permeant-carbonyl group interaction.

V. The diffusion of small nonelectrolytes through planar lipid bilayer membranes (egg phosphatidylcholine-decane) has been measured (Walter and Gutknecht 1986). It was found using an *electrical (membrane voltage) technique* to measure permeability that very small molecules (MW $<$ 50) diffused much faster than those with higher molecular weights, and the overall data were consistent with a solubility-diffusion model in the lipid bilayer (acting as a soft polymer), as the permeabilities were inversely related to molecular weight and strongly related to hydrophobicity of the solvent. In so far as the egg phosphatidylcholine-decane is a reasonable proxy for the BBB cell membranes, this experimental technique provides support to the notion that membrane potentials are important for BBB permeation.

VI. Given the importance of zwitterions (amino acids, some drugs, etc.) at physiological pH 7.4 levels, and the known effect of the membrane dipole potentials on permeation of cell membranes, *it can be concluded that the dipole moment is an important characteristic of the ability of drugs (or other physiologically important molecules) to cross the BBB.* To date, there appears to be no consideration in the literature of this factor.

8. The passive diffusion model (Eqs. 1–5) developed above was derived for alcohols and glycols from

methanol to 1-butanol, as the log *PS* data for higher alcohols are not available. However, there have been extensive studies of the effects of alcohols on cell membranes and the CNS of rats (Lyon and McComb 1981; McKarns et al. 1997; McCreery and Hunt 1978; Ingólfsson and Andersen 2011; Ho et al. 1994; Aagaard et al. 2006). There is no dispute that alcohols modulate lipid bilayer properties. There is a chain length effect on alcohol-induced modulation of lipid bilayers, often referred to as a "cut-off" effect (where the increasing potency with increasing chain length effects eventually levels off, or decreases). These modulations can be temporary or permanent. These "cut-off" effects are observed for many systems, from the formation of the photoactivated form of rhodopsin in 1-palmitoyl-2-oleyl-phosphatidylcholine lipid vesicles which occurs at chain length <6, the anaesthetic effects on tadpoles which reaches a maximum at C10, *to the ataxia (intoxication) effects on rats which maximizes at C6–C7* (Lyon and McComb 1981). To understand the molecular mechanisms which underpin Eq. 5, several studies using the PC lipid bilayer model membrane DPHYPC have been undertaken:

I. A physiochemical modification of the membrane protein–lipid interface is known to occur which is based on a hydrogen-bonding interaction between the alcohols and the phosphate moiety of the PC head of the membrane bilayer (Lyon and McComb 1981; McKarns et al. 1997; Chiou et al. 1991; Ho et al. 1994). A strong inverse relationship was found between the effective doses that produced ataxia and the membrane buffer partition coefficient (or log *P*) up to the cut-off point (McKarns et al. 1997; McCreery and Hunt 1978). The "cut-off" effect could also be due to steric effects between the alcohol and membrane bilayer. It was found that short-chain alcohols (1-hexanol and shorter) cause volume increases when partitioning into dimyristoylphosphatidylcholine bilayers, whereas longer alcohols cause volume decreases. These cut-off effects tend to appear when the alcohol chain length is approximately equal to half the acyl chain length of the bilayer-forming lipids (Aagaard et al. 2006).

II. The vesicle-forming lipid 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (DCPC) was used with a fluorophore to determine the bilayer-modifying potency (D*) of a series of alcohols (where D* is the concentration at which the alcohol doubled the quenching rate) (McKarns et al. 1997). The data are shown in Table 2 along with the calculated $\Delta G_{\text{octanol}}$ values for DPHYPC (DPHYPC is similar to DCPC but has a saturated C20 acyl chain instead of the C22 acyl chain with a double bond at C13–C14). *A linear relationship exists between* $-\Delta G_{\text{octanol}}$ *and D* up to the "cut-off" at 1-heptanol*. Conversely, a linear relationship exists between $-\Delta G_{\text{octanol}}$ and log D*:

$$-\Delta G_{\text{octanol}} = -0.40 \log \text{D}^* - 8.16 \left(R^2 0.881,\ \text{SEE } 0.18\right) \quad (11)$$

It is also clear that steric effects are active for the three alcohols which are not straight chain alcohols with a terminal hydroxyl group (2-propanol, 2-butanol and t-butyl alcohol), which are outliers to the 1-alcohol series. These data are consistent with a steric effect being responsible for the "cut-off" behaviour.

III. In a study of the ability of alcohols to toxically break down or create reversible graded increases in cell membrane permeability in rat liver epithelial cells, it was found that a linear QSAR relationship existed between log (1/LDH50) and log *P* as the measure of hydrophobicity. LDH or lactate dehydrogenase release is correlated with the breakdown or change in membrane permeability. The LDH50 values are defined as the concentrations which elicited a 50 % increase of LDH50 release relative to the untreated control (Lyon and McComb 1981). These data are shown in Table 2 along with $\Delta G_{\text{octanol}}$ values for DPHYPC. *A linear relationship exists between* $-\Delta G_{\text{octanol}}$ *and LDH50 up to the "cut-off" at 1-pentanol*. Conversely, a linear relationship exists between $-\Delta G_{\text{octanol}}$ and log LDH50:

$$-\Delta G_{\text{octanol}} = -0.32 \log \text{LDH50} - 6.99 \left(R^2 0.938,\ \text{SEE } 0.09\right) \quad (12)$$

IV. The passive diffusion model (Eqs. 1–5) was developed for alcohols up to C5 in length. It is clear from 8 II and III above that the "cut-off" point for alcohols permeating, or structurally modulating, the model PC lipid bilayer membranes is about C5–C6 in chain length. The log *PS* data are usually derived from rats, and the data discussed above are all consistent with the experimental ataxia finding in rats which showed a "cut-off" at C6–C7 in alcoholic chain length. These data are also consistent with the

Table 2 Comparison of diphytanolphosphatidylcholine (DPHYPC) and *n*-octanol properties in a series of alcohols

	ΔG_s DPHYPC	ΔG_s octanol	D^* (McKarns et al. 1997)	LDH50 (Lyon and McComb 1981)	Sum(+) charges DPHYPC	Sum(±) charges DPHYPC	ΔG_{CDS} DPHYPC
Gas	0	0			1.154		
Water	−71	−3.79					24.4
Methanol	−93.9	−9.21	666	3.4			
Ethanol	−91.7	−9.04	147	1.7	1.298	−3.402	−4.1
1-Propanol	−90.3	−8.89	35.5	0.47	1.295	−3.399	−3.9
2-Propanol	−89.9	−8.94	93.3	0.16			
1-Butanol	−88.5	−8.65	11.9	0.16	1.293	−3.396	−3.3
2-Butanol	−88	−8.72	23.7	0.36			
t-Butanol	−86.6	−87.2	47.3	0.19			
1-Pentanol	−87.4	−85.5	4.7	0.047	1.291	−3.392	−3.3
1-Hexanol	−85.5	−8.37	1.2	0.012	1.287	−3.389	−3.2
1-Heptanol	−84.1	−8.20	0.4	0.0045	1.284	−3.386	−2.8
1-Octanol	−82.3	−8.03	0.4	0.00089			−2.7
1-Nonanol	−80.1	−7.81	0.2		1.280	−3.382	−2.3
1-Decanol	−77.8	−7.61	0.6		1.276	−3.377	−2.0
1,2-Ethanediol	−76.1	−56.5			1.272	−3.372	

ΔG values in kcal/mol

See Eq. 10 which defines the relationship between ΔG_{DPHYPC} and $\Delta G_{Octanol}$

Sum(+) Charges DPHYPC are the atomic charges in volts on the N and P atom of the PC head group. Sum(±) charges are the sum of atomic charges on the N, PO_4 and the two oxygens of the two –OC(O)– groups of the acyl chains of DPHYPC. See Eqs. 13 and 14

D^* is the concentration at which the alcohol doubled the quenching rate of the fluorophore/1,2-dierucoyl-*sn*-glycero-3-phosphocholine (DCPC) system used to determine the bilayer-modifying potency (D^*) of a series of alcohols (McKarns et al. 1997): see Eq. 11

LDH or lactate dehydrogenase release is correlated with the breakdown or change in membrane permeability. The LDH50 values are defined as the concentrations which elicited a 50 % increase of LDH50 release relative to the untreated control (Lyon and McComb 1981); see Eq. 12

ΔG_{CDS} in kcal/mol, include hydrogen-bonding interactions, solute–solvent cavity interactions and other non-electrostatic solute–solvent effects: see Eq. 15

log *PS* model for the xanthines (see 1. above) which permeate the BBB by both passive and facilitated diffusion, and which showed an inverse relationship with molecular volume, (see Eq. 9) compared to that found for the smaller alcohols (see Eq. 4). This is consistent with the "cut-off" point seen for alcohols which is controlled by steric forces.

V. These "cut-off" change of mechanism effects for the alcohols-DPHYPC series could possibly be due to a dipole effect, rather than a steric effect which increases as the aliphatic chain length increases. To eliminate this possibility, the relationship between the atomic charges on the phosphate group of the PC moiety and the –C(O)O– of the acyl chain have been examined. The following linear relationships were observed: The straight chain aliphatic alcohols varied from methanol by one carbon at a time up to 1-decanol. Non-linear aliphatic chains such as 2-propanol, 2-butanol, tertiary-butanol and 2-methyl-1-propanol were clear outliers from the straight chain alcohol relationship. Sum(+) PC charges are the atomic charges in volts on the N and P atom of the PC head group. Sum(±) charges are the sum of atomic charges on the N, PO_4 and the two oxygens of the two –OC(O)– groups of the acyl chains of DPHYPC. *The sum (±) charges is proportional to the dipole moment of DPHYPC in the various alcohols*.

$$\text{Length carbon chain} = -203.29\ \text{Sum}(+)\ \text{PC charges} + 267.69\ (R^2\ 0.864,\ \text{SEE}\ 1.19) \quad (13)$$

or by omitting methanol as an outlier (probably due to its capacity to form multiple solvation interactions because of its smaller size)

$$\text{Length carbon chain} = -305.25\ \text{Sum}(+)\ \text{PC charges} + 398.61\ (R^2\ 0.942,\ \text{SEE}\ 0.33)$$

$$\text{Length carbon chain} = -0.0015\ \text{Sum}(+/-)\ \text{charges} + 6.0\ (R^2\ 0.273,\ \text{SEE}\ 2.74) \quad (14)$$

or

$$\text{Length carbon chain} = 270.27\ \text{Sum}(+/-)\ \text{charges} + 921.77(R^2\ 0.991,\ \text{SEE}\ 0.28)$$

if methanol is omitted as an outlier.

A linear relationship exists between the straight chain alcohols from ethanol to 1-decanol and the ΔG_{CDS} value in kcal/mol (which include hydrogen-bonding interactions between the alcohols and DPHYPC, the energy to create a cavity for the solute DPHYPC and other non-electrostatic solute–solvent interactions (Marenich et al. 2009)).

$$\text{Length carbon chain} = 0.73\Delta G_{CDS} + 7.59(R^2\ 0.050,\ \text{SEE}\ 3.13) \quad (15)$$

Or by omitting methanol as an outlier

$$\text{Length carbon chain} = 3.90\Delta G_{CDS} + 17.96(R^2\ 0.969,\ \text{SEE}\ 0.52)$$

These relationships clearly demonstrate that no dipolar interaction nor hydrogen bonding, nor cavity effects between the model membrane and the alcohols is responsible for the "cut-off" effect. The outliers (which all involve alcohols with known steric hindrance solvent effects) to Eqs. 13–15 also reinforce the conclusion that steric effects from incrementally increasing the aliphatic chain length (by one carbon at a time) are clearly responsible for the "cut-off "effect in these alcohols.

Analysis of Literature log *PS* QSAR Relationships

Recent studies of BBB permeability relationships (Carpenter et al. 2014; Liu 2005) have been reanalysed using the four factors identified in this study, ΔG_{water}, $\Delta G_{octanol}$, D and V. Liu 2005 examined how caffeine, theophylline, theobromine, fluoxetine, NFPS and CP-141938 permeated (log *PS*) the rat BBB and equilibrated in plasma. A physiologically based pharmacokinetic model was used to correlate an in vivo log *PS* with in situ log *PS* (R^2 0.83). The following relationships were found using Liu's 2005 data:

$$\log PS = -0.11\Delta G_{water} - 0.89(R^2 0.683,\ \text{SEE}\ 0.52)$$

$$\log PS = 0.11\Delta G_{octanol} - 0.836(R^2 0.434,\ \text{SEE}\ 0.69)$$

$$\log PS = -0.002V - 2.09(R^2 0.032,\ \text{SEE}\ 0.91)$$

$$\log PS = -0.28D - 1.15(R^2 0.588,\ \text{SEE}\ 0.59)$$

$$\log PS \sim 0.18\Delta G_{water} + 0.36\Delta G_{octanol} - 0.37D + 0.02V - 1.58(R^2 = 0.754,\ \text{SEE}\ 0.73)$$

Gratton (Liu et al. 2004) examined the relationship between log *PS* and antipyrine, 2-propanol, 95005, erythritol, mannitol, sucrose, thymine, ethanol, estradiol, thiourea, urea and ethylene glycol. The following relationships were found:

$$\log PS = -0.085\Delta G_{water} - 1.49(R^2 0.592,\ \text{SEE}\ 0.94)$$

$$\log PS = 0.097\Delta G_{octanol} - 1.43(R^2 0.434,\ \text{SEE}\ 1.10)$$

$$\log PS = 0.002V - 3.10\ (R^2 0.012,\ \text{SEE}\ 1.47)$$

$$\log PS = -0.08D - 2.53(R^2 0.012,\ \text{SEE}\ 1.47)$$

$$\log PS \sim 0.14\Delta G_{water} + 0.38\Delta G_{octanol} - 0.12D + 0.02V - 1.34(R^2 0.900,\ \text{SEE}\ 0.56)$$

Murikami (Liu et al. 2004) examined the relationship between log *PS* and digoxin*, hypoxanthine, methotrexate, phenylalanine**, quinidine*, theophylline, valproic acid, mannitol, sucrose, alanine**, cyclosporine A*, glibencamide, glucose**, iodoantipyrine, quinine, tolbutamide, vinblastine*, warfarin, cimetidine, vincristine* and thiourea. The compounds* were considered to be transported by P-gp, and the compounds ** were transported by uptake mechanisms. The following relationships were found, but actively transported cyclosporine, digoxin and vinblastin were excluded as clear outliers.

$$\log PS = -0.041\Delta G_{water} - 2.29(R^2 0.346,\ \text{SEE}\ 0.69)$$

$$\log PS = 0.043\Delta G_{octanol} - 2.39(R^2 0.247,\ \text{SEE}\ 0.74)$$

$$\log PS = 0.001V - 3.27(R^2 0.001,\ \text{SEE}\ 0.86)$$

$$\log PS = 0.078D - 3.58(R^2 0.053,\ 0.84)$$

$$\log PS \sim 0.13\Delta G_{water} + 0.27\Delta G_{octanol} - 0.11D + 0.01V - 2.67(R^2 0.564,\ \text{SEE}\ 0.63)$$

By excluding all the 8 compounds that were identified as being actively transported, the following relationships were found:

$$\log PS = -0.051\Delta G_{water} - 2.14(R^2 0.518,\ \text{SEE}\ 0.67)$$

$$\log PS = 0.060\ \Delta G_{octanol} - 2.03(R^2 0.410,\ \text{SEE}\ 0.75)$$

$$\log PS = 0.001V - 3.50\ (R^2 0.005,\ \text{SEE}\ 0.97)$$

$$\log PS = 0.157D - 4.20\ (R^2 0.197,\ 0.87)$$

$$\log PS = 0.15\Delta G_{water} + 0.33\Delta G_{octanol} + 0.13D + 0.01V - 2.84(R^2 0.895,\ \text{SEE}\ 0.37)$$

The multiple regression relationship has improved significantly with the exclusion of the 8 actively transported

compounds. (See "Experimental" section regarding the low R^2 values)

Liu et al. (2004) examined the relationship between log *PS* and 28 compounds (see Experimental).

$$\log PS = -0.036\Delta G_{\text{water}} - 2.09\left(R^2 0.314,\ \text{SEE } 0.71\right)$$

$$\log PS = 0.037\Delta G_{\text{octanol}} - 2.10\left(R^2 0.237,\ \text{SEE } 0.75\right)$$

$$\log PS = -0.002V - 2.42\left(R^2 0.073,\ \text{SEE } 0.82\right)$$

$$\log PS = -0.06D - 2.49\left(R^2 = 0.060,\ \text{SEE } 0.83\right)$$

$$\log PS \sim -0.128\Delta G_{\text{water}} - 0.124\Delta G_{\text{octanol}} - 0.001D - 0.002V - 2.26\left(R^2 0.374,\ \text{SEE } 0.72\right)$$

By excluding the 9 compounds that were identified as being actively transported, the following relationships were found:

$$\log PS = -0.054\Delta G_{\text{water}} - 1.82\left(R^2\, 0.399,\ \text{SEE } 0.72\right)$$

$$\log PS = 0.046\Delta G_{\text{octanol}} - 1.96\left(R^2 0.196,\ \text{SEE } 0.83\right)$$

$$\log PS = 0.001\ \text{V} - 2.42\left(R^2 0.073,\ \text{SEE } 0.82\right)$$

$$\log PS = 0.075D - 2.94\left(R^2 = 0.017,\ \text{SEE } 0.91\right)$$

$$\log PS \sim -0.055\Delta G_{\text{water}} + 0.038\Delta G_{\text{octanol}} + 0.101D + 0.006V - 2.77\left(R^2 0.678,\ \text{SEE } 0.58\right)$$

The multiple regression relationship has improved significantly with the exclusion of the 9 actively transported compounds (See "Experimental" section regarding the low R^2 values).

By comparing the regression equations derived for the alcohols (Eqs. 1–5), and the xanthines (Eqs. 6–9), with those derived from the Liu 2005, Gratton et al. 1997 data, it can be seen that a similar pattern emerges. The linear regression equations are more accurate than the multiple regression equations which have less than the optimal data points to be robust. There is a negative dependence on the desolvation from water, a positive dependence on the lipophilicity, and smaller dependencies on molecular volume and dipole moment. The literature relationships are for a diverse and wide range of permeants that tend to cloud systematic structural change (similar to those in linear free energy relationships) which is seen for the alcohols and xanthines series which are more closely structurally comparable. It is clear that including actively transported species in the regression relationships lowers the correlations, indicating that these species are really outliers, or at best increase scatter. Based on the observations above for the actively transported stereoisomers (Sect. 6 above), *it is clear that where any significant binding interaction with transport proteins in the BBB occurs, then no simple relationship with log PS is easily distinguishable*.

It should be noted that some of the literature log *PS* values are for compounds which exist as charged ions or zwitterions at physiological pH 7.4: these compounds were treated as the solvated neutral species in deriving the regression equations. This implies that it is the desolvation of the neutral species that controls the kinetics, since the very large ΔG_{water} values for these charged species would effectively preclude any initiation of the permeation process. This situation is possible since there would be both solvated charged and neutral species at pH 7.4.

Conclusions

The permeability of the BBB is dependant on desolvation, lipophilicity, molecular volume and dipole moment. Previous models for BBB permeability have not considered desolvation and dipole moment as critical factors. The model applies to passive diffusion processes, and some facilitated diffusion processes. Passive diffusion transport processes for many common drugs appear to be less common than active transport processes, so BBB permeability models for passive transport may not apply to active transport processes, particularly where complex membrane protein binding processes (e.g. stereoselectivity) are involved. Model phosphatidylcholine (PC) lipid bilayer membranes have been used to evaluate how charged or polar neutral compounds can interact through their molecular dipoles with the cell membrane to induce electromechanical changes in the cell membrane which facilitate permeation. The free energy of solvation in *n*-octanol has been shown to be a good measure of membrane lipophilicity by calculating the solvation free energy of a model PC lipid membrane in a series of closely related alcohols. The passive diffusion model for alcohols has been shown to correlate with previous studies of the modulation of membrane bilayers by alcohols which showed a "cut-off" point in potency, which is related to molecular size. The dominant species at physiological pH levels in blood serum is integrated into the model, and particularly affects desolvation energies for charged and zwitterionic species. For most drugs and related molecules, the neutral species are the permeating species.

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