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Theoretical Aspects of P-glycoprotein Mediated Drug Efflux on the Distribution Volume of Anaesthetic-related Drugs in the Brain

R. N. UPTON*

Department of Anaesthesia and Intensive Care, Royal Adelaide Hospital, University of Adelaide, Adelaide, South Australia

SUMMARY

P-glycoprotein in the membranes of endothelial cells actively transports some drugs out of the brain.

The theoretical effect of P-glycoprotein mediated drug efflux on the cerebral distribution volumes of drugs was examined, with particular emphasis on anaesthetic-related drugs (often characterized by moderate to high permeability across the blood brain barrier due to their lipophilicity and intermediate molecular weight). An analytical equation for the cerebral distribution volume in the presence of the efflux was derived, and validated by modelling the same system using differential equations.

The efflux was shown to lower both the membrane and intracellular drug concentrations in parallel, and to reduce the time required for brain:blood equilibration. The net effect of the efflux was governed by the ratio of the P-glycoprotein drug clearance from the membrane (Pcl) and the permeability of the membrane (PS). It was therefore a balance between the rate that a drug could be pumped out of the membrane by the efflux system, and the rate that the drug leaked back in due to the permeability of the membrane for the drug. The effect of the efflux was therefore more pronounced for drugs with membrane-limited cerebral kinetics (e.g. morphine), but was nevertheless significant for drugs with more flow-limited kinetics (e.g fentanyl). The cerebral distribution volume was also influenced by the free fraction in blood and the free fraction in the intracellular space in the conventional manner.

There are no theoretical limitations to the P-glycoprotein system influencing the cerebral distribution volume of moderately lipophilic anaesthetic-related drugs.

Key Words: BRAIN: cerebral distribution volume. PHARMACOKINETICS: P-glycoprotein

P-glycoprotein (also known as the multidrug transporter) is normally expressed in the epithelial cells of the liver, kidney, intestine and the endothelial cells of the blood-brain barrier[1]. It consumes ATP and actively removes compounds from the membrane of cells directly into the adjacent lumen. P-glycoprotein substrates are generally moderately lipophilic, basic (or uncharged), and have a molecular weight (MW) between approximately 250 and 1900 Da[1]. An analogous protein of the same family (multi-drug resistance protein, MRP) may serve a similar role (amongst others) for lipophilic, acidic compounds[2].

Functionally, P-glycoprotein-mediated efflux serves to limit exposure to xenobiotic compounds by reducing their rate of oral absorption, increasing their rate of excretion by the liver and kidney, and reducing their entry into the brain. The latter organ is of particular interest in the field of anaesthesia, as some anaesthetics and analgesics (e.g. morphine[3], midazolam[4], fentanyl[5], methadone[6]) that have direct effects on the central nervous system are P-glycoprotein substrates.

The role of P-glycoprotein mediated drug efflux in moderating the brain concentrations and central nervous system (CNS) effects of drugs is still emerging, but knowledge is advancing rapidly due to the ability to "knock-out" (remove) the gene for this and related proteins in mice, and to the development of specific P-glycoprotein inhibitors. P-glycoprotein knockout mice are apparently normal except for a considerably enhanced sensitivity to some drugs[1]. The analgesic effect of morphine (via a hot plate method) was shown to be enhanced approximately four-fold in knockout mice, while that of methadone was substantially increased and that of fentanyl was increased to

*Ph.D., Principal Medical Scientist, Department of Anaesthesia and Intensive Care, Royal Adelaide Hospital, University of Adelaide, Adelaide, South Australia.

Address for reprints: Dr R. N. Upton, Principal Medical Scientist, Department of Anaesthesia and Intensive Care, Royal Adelaide Hospital, University of Adelaide, North Terrace, Adelaide, S.A. 5005.

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a small extent[6]. The effects of pethidine and morphine-6-glucuronide were unchanged. There is good evidence that these analgesic changes are mediated by changes in the brain concentrations of the drugs. The brain concentrations of morphine have been shown to be two- to five-fold higher when P-glycoprotein was absent or inhibited[3,7,8].

These findings are consistent with P-glycoprotein mediated drug efflux reducing the cerebral distribution volumes of substrate drugs. The accepted explanation for the determinants of the cerebral distribution volume (V_{brn}) is based on diffusion of the free form of the drug across a blood-brain barrier, which excludes bound (and ionised) forms of the drug. If the capillary volume of the brain is V_{cap}, and the real intracellular volume of the brain is V_i, then V_{brn} is given by the following equation or its derivatives[9,10]:

$$V_{brn}=V_{cap}+V_i\frac{f_{u,p}}{f_{u,i}} \quad (1)$$

The free (unbound) fractions in plasma and the intracellular space are represented by $f_{u,p}$ and $f_{u,i}$ respectively. Although it is the free concentration of drug in the brain that is thought to govern cerebral drug effects, the total cerebral distribution volume of anaesthetic-related drugs is important as it governs the time required for the cerebral drug concentrations to equilibrate with the drug concentration in arterial blood. There is evidence that differences in this equilibration time dictate some important clinical properties of intravenous anaesthetics[11] and opioids[12].

The aim of this paper is to modify Equation 1 to account for P-glycoprotein mediated drug efflux from the brain. This is intended to provide insight into the determinants of the cerebral distribution volume in the presence of such efflux, and to provide a theoretical foundation for subsequent experimental work in this area.

MATERIALS AND METHODS

Equation 1 is derived by equating the input and output rates of drug transport for a model system as shown in Figure 1. At steady state, these rates are equal by definition. To add the effect of P-glycoprotein mediated efflux requires some modification of this system, as shown in Figure 2. As P-glycoprotein removes drug directly from the cell membrane[1], it is necessary for the model to represent the membrane concentration of the drug. For both model systems, the capillary, membrane and intracellular volumes are assumed to be well mixed, and can therefore be represented by compartments.

Venous Concentrations at Steady State

When the system shown in Figure 2 is at steady state, it is evident that the venous concentration in the capillary compartment (C_v) is equal to the arterial concentration (C_{art}) entering the compartment:

$$C_v=C_{art} \quad (2)$$

Intracellular Concentrations at Steady State

Furthermore, the intracellular drug concentration (C_i) at steady state can be derived from equating the rate that drug is entering the intracellular compartment ($rate_{in}$) with the rate that it is leaving the compartment ($rate_{out}$):

$$rate_{in}=rate_{out} \quad (3)$$

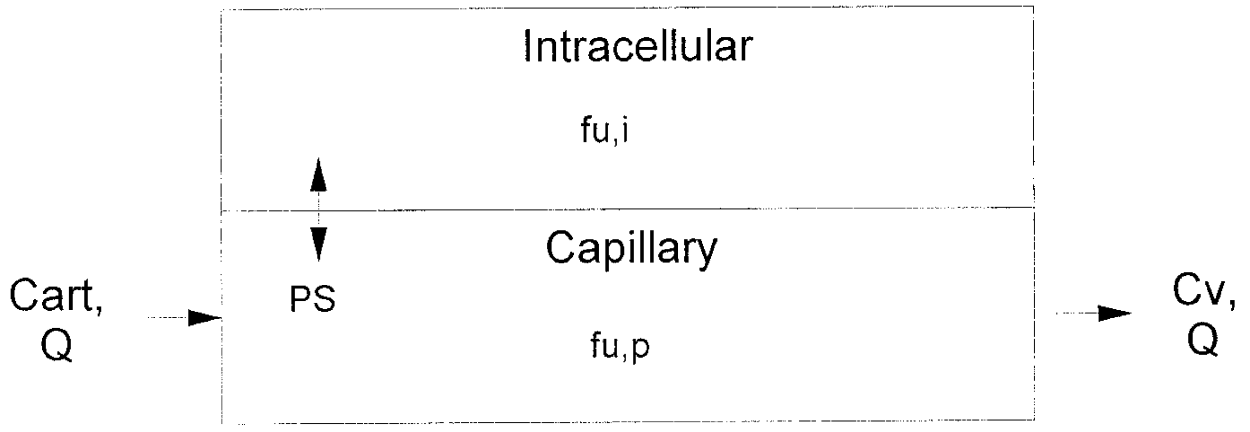

FIGURE 1: A traditional membrane-limited model of drug kinetics in an organ. The membrane barrier has no capacity to hold drug, and the permeability of the membrane is given by the permeability-surface area coefficient PS, which has the units of volume per unit time. Drug is delivered to the capillary volume at a rate given by the arterial concentration (C_{art}) multiplied by the organ blood flow (Q). Drug leaves the organ in venous blood at a rate given by C_v.Q, where C_v is the venous drug concentration. The free fraction in plasma and the intracellular space are given by $f_{u,p}$ and $f_{u,i}$ respectively.

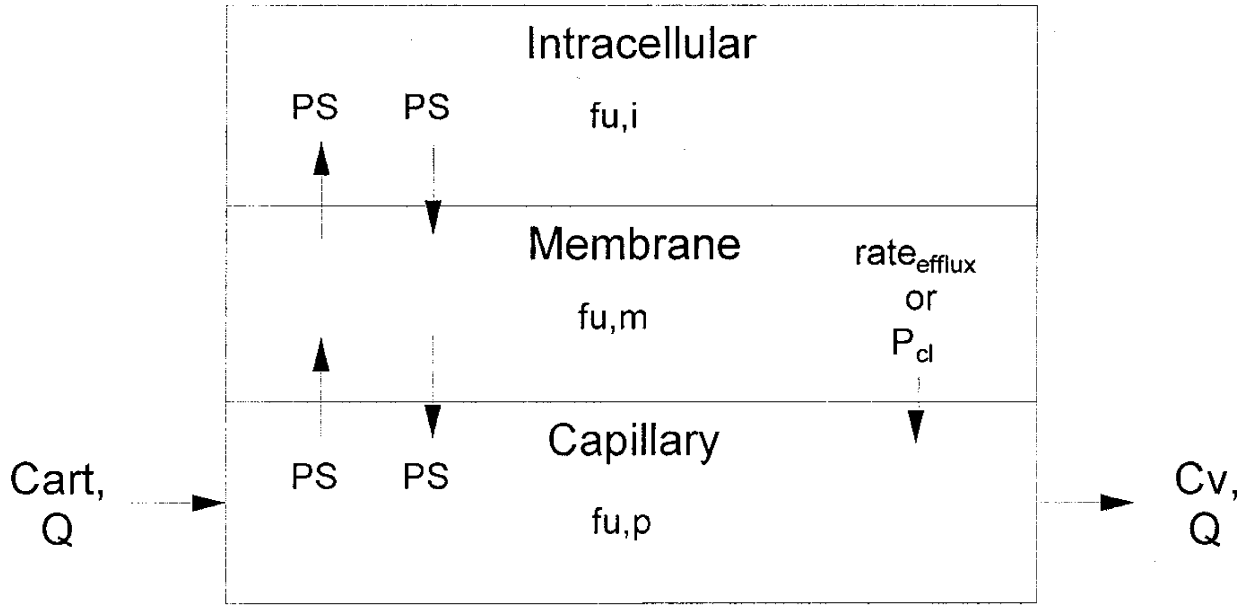

FIGURE 2: The model system shown in Figure 1 has been modified to account for P-glycoprotein mediated efflux from the cell membranes. There are now distinct permeability terms for drug entering and leaving the membrane—algebraic manipulation will show that PS in this system is equivalent to PS in the system shown in Figure 1. The drug concentration in the membrane can be represented by giving the membrane a finite volume. Drug can be actively transported from the cell membrane at a rate indicated by the general term "$rate_{efflux}$", which in specific cases is referred to as P_{cl}. The free fraction in the membrane is given by $f_{u,m}$.

If C_m is the concentration of the drug in the membrane, Equation 3 becomes:

$$PS.f_{u,m}.C_m = PS.f_{u,i}.C_i \quad (4)$$

where $f_{u,i}$ is the free fraction in the intracellular space, while $f_{u,m}$ is the "free fraction" of the drug in the cell membrane. The meaning of $f_{u,m}$ will be discussed later. "PS" is the permeability of the membrane (Figure 1), and is a term with the units of flow (volume per unit time). It can be thought of as the proportionality constant relating the total rate of drug diffusion (mass per unit time) and the free drug concentration (f_u.C). Rearranging to find the ratio of C_i over C_m:

$$\frac{C_i}{C_m} = \frac{f_{u,m}}{f_{u,i}} \quad (5)$$

Membrane Concentrations at Steady State

As for the intracellular concentrations, the drug concentration in the membrane of the system shown in Figure 2 can be derived from equating the rate that drug is entering the membrane ($rate_{in}$) with the rate that it is leaving ($rate_{out}$). However, the rate of efflux of drug from the membrane by P-glycoprotein ($rate_{efflux}$) must also be accounted for. Again, the sum of these fluxes will be equal at steady state.

$$rate_{in} = rate_{out} + rate_{efflux} \quad (6)$$

Expressing Equation 6 in terms of concentrations and permeability terms (as was done for Eqn. 4):

$$PS.f_{u,p}.C_v + PS.f_{u,i}.C_i = 2.PS.f_{u,m}.C_m + rate_{efflux} \quad (7)$$

Substituting Equation 5 for C_i and solving for C_m gives:

$$Cm = \frac{f_{u,p}}{f_{u,m}}.Cv + \frac{rate_{efflux}}{PS.f_{u,m}} \quad (8)$$

Special Cases of the General Equation

Equation 8 specifies the rate of P-glycoprotein mediated efflux from the membrane in general terms. If the efflux is mediated by a single population of energy dependent carrier proteins, the rate of efflux is given by the following modification of the Michaelis-Menten equation:

$$rate_{efflux} = \frac{V_{max}.f_{u,m}.C_m}{(k_m + f_{u,m}.C_m)} \quad (9)$$

V_{max} is the maximum rate of efflux possible, and k_m is the free concentration at which half of the maximum rate is achieved. Equation 9 can be substituted into Equation 8, but solving for C_m gives equations that are intractably large. However, by considering the case where the C_m is much less than

k_m (i.e. pseudo first order kinetics), the rate of efflux can be simplified to:

$$rate_{efflux} = \frac{V_{max}}{k_m} f_{u,m}.C_m \quad (10)$$

V_{max}/k_m is the apparent clearance of the drug from the membrane, and will be defined as the P-glycoprotein-mediated membrane clearance of the drug (P_{cl}). It is the proportionality constant between the rate of efflux and the free drug concentration in the membrane. Substituting Eqn. 10 into Eqn. 8 and rearranging to give C_m over C_v gives:

$$\frac{C_m}{C_v} = \frac{1}{\left(1 + \frac{P_{cl}}{PS}\right)} \cdot \frac{f_{u,p}}{f_{u,m}} \quad (11)$$

Effect of P-glycoprotein Efflux on Cerebral Distribution Volume

For a given constant afferent arterial drug concentration, Equations 2, 5 and 11 give the corresponding concentrations in venous (capillary), intracellular and membrane compartments at steady state. To calculate the total apparent distribution volume of a drug in the brain requires assigning true volumes to these compartments. In a 70 kg man, the real volume of the brain (V_{real}) is approximately 1.3l and cerebral blood flow is approximately 0.75 l/min[13]. The capillary volume of the brain is approximately 4% of its total volume[14]. The total lipid content of the brain is relatively low, with an average value of 11% reported[15]. In the present model, the simplifying assumption is made that all this lipid separates the capillary and intracellular spaces. The remaining 85% of the brain is predominately water (78%) and protein (7%)[15], which will be attributed to the intracellular space. Based on these values, the apparent volume of distribution of the brain is the total amount of drug in the brain (the sum of concentration and volume in each compartment) divided by the afferent concentration, and approximates to:

$$V_{brn} \approx \frac{V_{real}*0.04*C_{art} + V_{real}*0.11*C_m + V_{real}*0.85*C_i}{C_{art}} \quad (12)$$

Substituting equations 5 and 11 and simplifying gives:

$$V_{brn} \approx V_{real} \left(0.04 + \frac{0.11}{\left(1 + \frac{P_{cl}}{PS}\right)} * \frac{f_{u,p}}{f_{u,m}} + \frac{0.85}{\left(1 + \frac{P_{cl}}{PS}\right)} * \frac{f_{u,p}}{f_{u,i}} \right) \quad (13)$$

where the term in brackets to the right of V_{real} corresponds to the brain:blood partition coefficient of the drug, often abbreviated to R. (In equations 12 and 13, the alternative symbol for multiplication (*) is used, to avoid confusion with decimal points.)

Independent Validation of Equations

To validate the analytical derivation of the equations derived above, the system shown in Figure 2 was written as differential equations and solved using a modelling program (Scientist for Windows, Version 2, Micromath, Utah, U.S.A.). The equations used are given in Appendix 1.

The venous (capillary), membrane and intracellular concentrations at steady state resulting from a constant arterial concentration entering the organ were simulated. Common parameter values were used for both the equations and the simulation, and the predicted concentrations at each site were compared. A discrepancy between the two could indicate an error in either.

Properties of the Equations

The effects of P-glycoprotein clearance (P_{cl}) on the concentration of drug in the membrane and intracellular compartments were examined using Equations 5 and 11. The concentration ratio was plotted against a range of values of P-glycoprotein clearance (P_{cl}) over membrane permeability (PS).

The effect of free fraction in the capillary, membrane and intracellular compartments on the cerebral distribution volume was examined using Equation 13. The free fraction in each compartment was in turn set at values ranging between 0 and 1, while the free fractions in the other compartments were set at 0.5. The ratio of P_{cl}/PS was also set at 0, 1 and 10. The cerebral distribution volume was calculated for each unique set of parameter values and the resulting data were plotted.

Sensitivity Analysis

The model based on differential equations was also used to simulate the effect of the extent of P-glycoprotein clearance (P_{cl}) and the permeability of the blood brain barrier (PS) on the cerebral kinetics of a hypothetical drug. The kinetics were summarized by two terms—the equilibrium brain:blood partition coefficient (R), and the time required for brain: blood equilibration to be complete. The permeability was set at three values relative to cerebral blood flow (Q), which were representative of membrane limited (PS=0.2.Q), partially membrane limited (PS=2.Q) and flow-limited (PS=20.Q) cerebral kinetics.

RESULTS

The solutions of the analytical equations and the model based on differential equations were identical, thereby independently confirming the validity of the equations as a description of the model system shown in Figure 2.

Equation 11 predicts that the steady state concentration of the drug in the membrane is influenced by the ratio of the P-glycoprotein clearance from the membrane (P_{cl}) and the permeability of the membrane (PS). As may be expected, the steady state membrane concentration is thus a balance between the rate that drug can be pumped out of the membrane by the efflux system, and the rate that the drug leaks back in due to the permeability of the membrane for the drug. The influence of P_{cl}/PS on the steady state drug concentrations in membrane and intracellular fluid (expressed as a ratio of the afferent arterial drug concentration) is shown in Figure 3. In this example, a 50% reduction was achieved when the ratio was 1, and the concentration was approximately 10% of normal when the ratio was 10. The asymptotic nature of the relationship thereafter produced only modest reductions in concentration for large increases in the activity of the efflux system.

Reductions in the membrane drug concentration produced parallel changes in the intracellular drug concentrations, as governed by Equation 5. Thus, the P-glycoprotein mediated efflux from the membrane had a major effect on the total brain concentration of a drug.

The effect of free fraction in each of the three tissue compartments on cerebral distribution volume is summarized in Figure 4. Decreasing free fraction in the plasma produced linear reductions in cerebral distribution volume, as also inherent in Equation 1. Increasing the P-glycoprotein membrane clearance (increasing Pcl/PS) reduced the slope of this line.

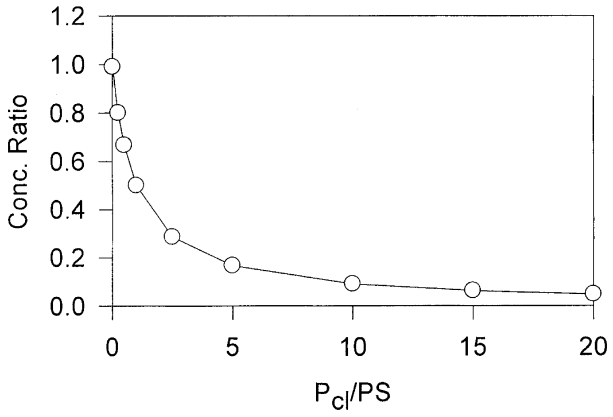

FIGURE 3: The effect of increasing the ratio of P-glycoprotein membrane clearance (P_{cl}) over membrane permeability (PS). The steady state drug concentration in membrane and intracellular fluid are shown, which are equal for this case where the free fraction in the membrane and intracellular space were the same. These concentrations are expressed as a ratio of the afferent arterial concentration—the ratio is 1 in the absence of P-glycoprotein mediated efflux (P_{cl}/PS = 0).

A novel contribution of the present work is the influence of the free fraction in the membrane on the cerebral distribution volume. Interestingly, this effect was predicted to be minor, as the total lipid content of the brain is relatively small, and this minimizes the contribution of the membrane concentrations to the total amount of drug in the brain. As expected however, the distribution volume was reduced in the presence of membrane efflux.

The effect of altered intracellular free fraction was as predicted by Equation 1—as free fraction is lowered to less than that in plasma, there are disproportionally larger increases in cerebral distribution volume. Small changes in intracellular free fraction for a drug that is highly bound in the intracellular space can therefore produce large changes in cerebral distribution volume. Again, increasing the P-glycoprotein membrane clearance (increasing P_{cl}/PS) reduced the magnitude of this effect. Indeed, the overall trend is that a high P-glycoprotein membrane clearance greatly reduced the sensitivity of the cerebral distribution volume to the free fraction in both plasma and intracellular fluid.

The results of the sensitivity analysis are summarized in Table 1. Membrane and intracellular concentrations were considered, but the data were similar and only data for the intracellular concentrations are shown for simplicity. This is consistent with the earlier observation that the membrane and intracellular concentrations change in parallel. Note that in the absence of P-glycoprotein efflux, increasing the degree of membrane limitation dramatically increases the time to equilibration, but the partition coefficient is unchanged. Increased P-glycoprotein clearance (P_{cl}) reduces both the brain:blood partition coefficient, and the time to reach brain to blood equilibration.

DISCUSSION

Methods for Measurement of Cerebral Distribution Volumes

While there are a number of methods for measuring the cerebral uptake of drugs, not all measure the cerebral distribution volume. In particular, methods based on rapid injection into the carotid artery (such as the brain uptake index method[16]) measure the acute extraction of drug across the brain which will be

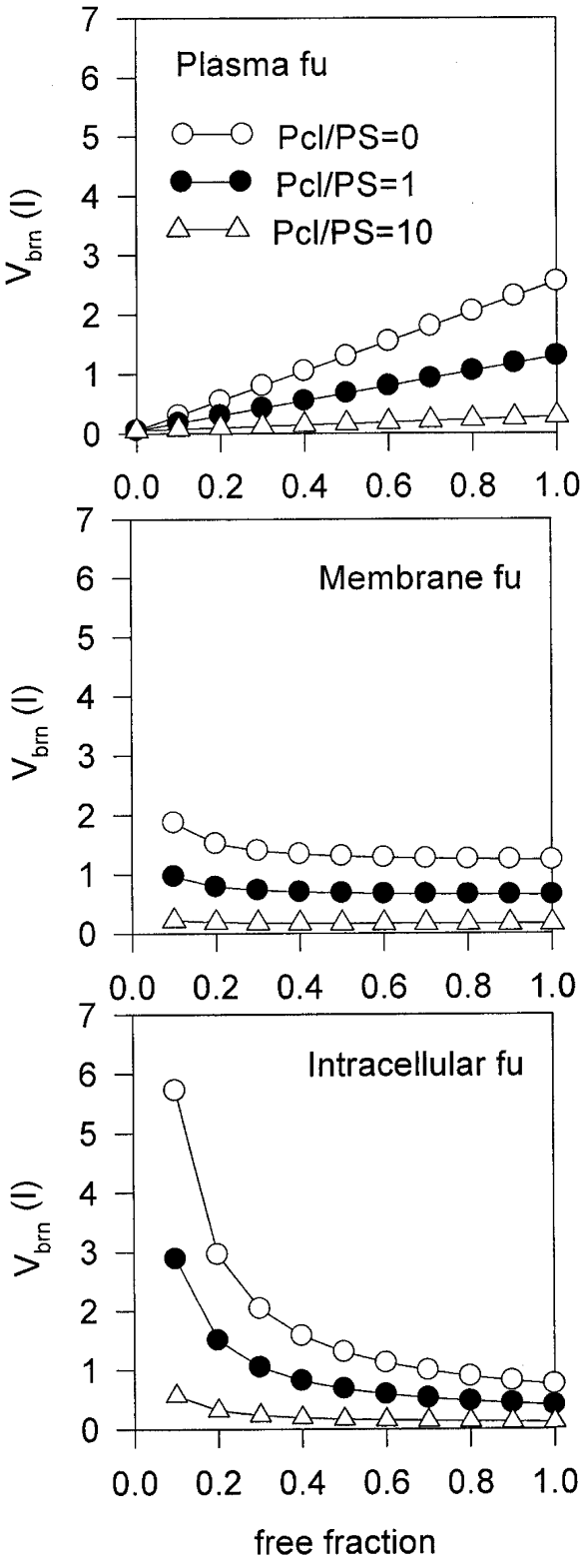

FIGURE 4: The effect of altering free fraction (fu) in plasma (top panel), the membranes (middle panel) or intracellular space (bottom panel) on distribution volume in the brain as given by Equation 13. The default free fraction for each site was 0.5. Data are shown for the cases of P_{cl}/PS set at 0, 1 and 10.

determined by blood-brain barrier permeability rather than steady-state distribution volume. In contrast, cerebral distribution volume can be inferred from the time-course of the arteriovenous drug concentration difference across the brain, as has been done using a chronically instrumented sheep preparation[11,17]. Furthermore, cerebral kinetics can be inferred, with some assumptions, from the time-course of cerebral drug effects and arterial drug concentrations in some instances[3].

Effect of P-glycoprotein Efflux on Cerebral Distribution Volume

An important implication of Equation 11 is that the net effect of P-glycoprotein clearance on the concentration of drug in the membrane is a balance between the rate at which drug is pumped out of the membrane, and the rate that it leaks back through the membrane due to the permeability of the membrane for the drug. The net effect is therefore highly dependent on the intrinsic permeability of the blood brain barrier to a drug. This is illustrated in Table 1.

The case of membrane-limited kinetics shown in Table 1 would be representative of drugs that cross the blood brain barrier very slowly—e.g. the glucuronides of morphine[18]. It was predicted that relatively low rates of P-glycoprotein efflux (P_{cl}) could produce substantial reductions in the brain blood partition coefficient of these compounds, and this is supported by in vitro evidence that P-glycoprotein activity contributes to the blood:brain barrier for morphine-6-glucuronide[19]. The partially membrane-limited case in Table 1 would be repre-

TABLE 1

The effect of the extent of membrane limitation in cerebral kinetics (see text) and the effective clearance of drug from the blood brain barrier by the P-glycoprotein system (P_{cl} in l/min) on 1) the steady state brain:blood partition coefficient (R) and 2) the total time required for brain:blood equilibration

	Membrane limited	Partially membrane limited	Flow limited
Brain: blood partition coefficient			
P_{cl} = 0	1	1	1
P_{cl}=1	0.13	0.6	0.94
P_{cl}=5	0.03	0.23	0.75
Time to brain: blood equilibrium (min)			
P_{cl} = 0	490	102.5	65
P_{cl}=1	166	64.5	61
P_{cl}=5	63	32	48.5

Data refer to the intracellular concentrations of hypothetical drugs for which the free fractions were fixed at the following values: $f_{u,p}$=0.5, $f_{u,m}$=0.1 and $f_{u,i}$=0.5. Cerebral blood flows and the real volumes of the capillary, membrane and intracellular spaces were also fixed at the values described in the text.

sentative of morphine, based on unpublished data (Upton et al) on its cerebral kinetics in sheep. The predicted fourfold reduction in brain:blood partition coefficient with the highest rate of P-glycoprotein activity (P_{cl}=5) is in keeping with the two- to five-fold reduction in brain concentrations and analgesia reported for morphine[3,7,8]. Note that the brain: blood equilibration time was also predicted to be reduced to a similar extent.

The flow-limited case in Table 1 would be representative of pethidine and alfentanil, which are known to have flow-limited cerebral kinetics[17]. For these drugs, it was predicted that increasing the P-glycoprotein efflux would produce modest reductions in both the partition coefficient and the time to equilibration. This strategy would therefore be an inefficient investment of metabolic energy. It is interesting to note that there is evidence that the analgesic effects of pethidine are not moderated by P-glycoprotein[6], while there have been no reports that alfentanil is a P-glycoprotein substrate. Fentanyl is an interesting case, as there is evidence that despite its relatively high lipophilicity, it has partially membrane limited kinetics in some organs[20]. The model would therefore predict that the effect of P-glycoprotein would be intermediate between the cases of morphine and pethidine discussed above. This is supported by the observation that the analgesia produced by fentanyl is increased in mice without P-glycoprotein, but to a lesser extent than for morphine[6]. There is also data that has been interpreted as active uptake of fentanyl into the brain (i.e. in the opposite direction to the P-glycoprotein efflux)[21].

Effect of Inhibition of P-glycoprotein

As a number of compounds are known to be inhibitors of P-glycoprotein, it is worthwhile considering the clinical potential of such drugs to increase the therapeutic index of anaesthetic and analgesic drugs that are P-glycoprotein substrates. As these drugs act in the brain, inhibition of P-glycoprotein efflux (lower value for P_{cl}/PS) would increase the brain concentrations expected for a given dose, allowing a reduction in dose for the same clinical effect. Those dose-dependent adverse effects (e.g. on some components of the cardiovascular system) that are not mediated by the drugs' cerebral concentrations would therefore also be reduced. For this effect to be useful, the ideal inhibitor should have no adverse effects of its own. No doubt the clinical potential of this strategy will emerge as more in vivo studies are done in this area.

Effect of Lipophilicity and Molecular Weight on Cerebral Distribution Volume

While molecular weight does not influence the cerebral distribution volume in theory, it is known that membrane permeability (PS) is inversely related to MW to the power of 3 or 4[22]. In practical terms, drugs with a MW of greater than 700 Da diffuse so slowly through the blood-brain barrier that they are effectively excluded from the brain[1]. High MW lipophilic drugs are problematic as they can have both high concentrations and long turnover times in the membrane, and have the potential to disrupt membrane function. It is has been suggested that removal of these compounds is an important role of the P-glycoprotein system[22].

Lipophilicity affects two important but unrelated aspects of cerebral drug distribution—membrane permeability and cerebral distribution volume. Membrane permeability (PS) is proportional to lipophilicity, and this dictates whether cerebral kinetics are flow- or membrane-limited. Once permeability becomes sufficiently high, the flow-limited cerebral kinetics predominate and further increases in the lipophilicity do not produce greater rates of blood: brain equilibration. The upper rate of equilibration is then governed by cerebral blood flow and the cerebral distribution volume.

The role of lipophilicity in determining cerebral distribution volume is made clear by considering the determinants of the free fraction of the drug in the membrane ($f_{u,m}$). In pure lipid, there are no protein molecules, so the free fraction is not determined by binding to macromolecules in the normal sense. Rather, the free fraction is equivalent to the activity rather than concentration of the drug in the lipid, and will by definition be inversely related to the lipid : pH 7.4 buffer partition co-efficient (P) of the drug:

$$f_{u,m} = \frac{1}{P} \qquad (14)$$

Substituting Eqn. 14 into Eqn. 11 shows that, as expected, highly lipophilic drugs (high P) can achieve high relative concentrations directly in the membranes and lipid components of the brain. However, the present study suggests that because the total lipid content of the brain is relatively low, the cerebral distribution volume (V_{brn}) is relatively unaffected by changes in lipophilicity alone (Eqn. 12).

In support of these relationships between lipophilicity and cerebral kinetics, it has been observed that homologous series of barbiturates of increasing lipophilicity are characterized by increasing membrane permeability in their uptake into the brain[23].

However, unbound distribution volume in the brain was little affected by changes in lipophilicity. Furthermore, changes in lipophilicity were also associated with concurrent changes in plasma protein binding, which had a much greater effect on the cerebral distribution volume. This may be the main mechanism by which the lipophilicity of a drug affects its distribution volume in the brain.

In conclusion, the system shown in Figure 2 is the simplest that accounts for the influence of P-glycoprotein on cerebral drug distribution, and its properties should be understood for this reason. In future work, this system will be compared with published experimental data on the cerebral distribution volumes of a number of anaesthetic-related drugs measured in a chronically instrumented sheep preparation. While the system may evolve as it is compared with these data, the present analysis will provide a framework on which to build the analysis.

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Appendix 1—Differential equations

The differential equations describing the system shown in Figure 2 and used simulations in the paper are listed below.

$$V_{cap}.C_v/dt = Q.(f_{up}.C_{art} - f_{up}.C_v) + PS.(f_{um}.C_m - f_{up}.C_v) + P_{cl}.f_{um}.C_m$$

$$V_m.C_m/dt = PS.(f_{up}.C_v + f_{ui}.C_i - 2.f_{um}.C_m) - P_{cl}.f_{um}.C_m$$

$$V_i.C_i/dt = PS.(f_{um}.C_m - f_{ui}.C_i)$$

Independent Variable

t = time

Dependent Variables

C_v = drug concentration in capillary blood

C_m = drug concentration in lipid membranes

C_i = drug concentration in the intracellular space

Parameters

V_{cap} = capillary blood volume
V_m = lipid volume of brain
V_i = intracellular volume of brain
f_{up} = free fraction in blood
f_{um} = free fraction in lipid membranes
f_{ui} = free fraction in intracellular space
PS = permeability of membrane barrier
P_{cl} = effective clearance of drug from the membranes by P-glycoprotein.