Chapter 7
Pharmacokinetic Concepts in Brain Drug Delivery

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Abstract This chapter presents the pharmacokinetic principles of blood-brain barrier (BBB) transport and the intra-brain distribution of small molecular drugs, in order to provide a basis for understanding drug delivery to the brain from a clinically relevant perspective. The most important concentrations to measure when determining drug distribution are those of the unbound drug, because it is the unbound drug that causes the pharmacological effect by interacting with the target. Therefore, this chapter also discusses the pharmacokinetic basis, the kind of information provided, and the in vivo relevance of the methods used to obtain reliable, therapeutically useful estimates of brain drug delivery. The main factors governing drug distribution to the brain are the permeability of the BBB to the drug (influx clearance), the extent of nonspecific binding to brain tissue, and the efflux clearance of the drug. The ratio of the influx and efflux clearances provides an estimation of the extent of drug equilibration across the BBB, described by the partition coefficient of unbound drug, $K_{p,uu,brain}$. This parameter is important, as active uptake and/or efflux transporters influence the brain concentrations of unbound drug in relation to those in plasma. The advantage of using $K_{p,uu,brain}$ during the drug discovery process lies in its ability to predict the potential success of drugs intended for action within the brain or, conversely, of those with few or no side effects in the brain.

Keywords Rate · Extent · Kp,uu · Interstitial · Intracellular · Species

Abbreviations

$[plasma]_u/[brain]_u$	Ratio of plasma to brain unbound drug concentrations
A_{brain}	Amount of drug per g brain tissue excluding blood
A_{slice}	Amount of drug per g of brain slice
$A_{tot.brain\_inc\_blood}$	Amount of drug per g brain tissue including blood
$AUC_{tot,brain}$	Area under the total brain concentration-time curve

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$AUC_{tot,plasma}$	Area under the total plasma concentration-time curve
$AUC_{u,brainISF}$	Area under the unbound brain ISF concentration-time curve
$AUC_{u,plasma}$	Area under the unbound plasma concentration-time curve
BBB	Blood-brain barrier
BBMEC cells	Bovine brain microvessel endothelial cells
BCSFB	Blood-cerebrospinal fluid barrier
Caco-2	Human epithelial colorectal adenocarcinoma cells
C_{buffer}	Concentration of drug in the buffer (brain slice method)
C_i	Apparent concentration of drug in a peripheral brain compartment i
CL_{act_efflux}	Active efflux clearance from brain to blood at the BBB (µl/min/g_brain)
CL_{act_uptake}	Active uptake clearance from blood to brain at the BBB (µl/min/g_brain)
CL_{bulk_flow}	Clearance by bulk flow from brain ISF to CSF (µl/min/g_brain)
CL_i	Intercompartmental clearance between brain ISF and the peripheral brain compartment i
CL_{in}	Net influx clearance of drug to the brain (µl/min/g_brain), also called permeability clearance
$CL_{metabolism}$	Metabolic clearance of drug in the brain or at the BBB (µl/min/g_brain)
CL_{out}	Net efflux clearance of drug from the brain (µl/min/g_brain)
$CL_{passive}$	Passive diffusional clearance of drug at the BBB
CNS	Central nervous system
CSF	Cerebrospinal fluid
$C_{tot,blood}$	Total concentration of drug in blood
$C_{tot.plasma}$	Total concentration of drug in plasma
$C_{u,brainISF}$	Concentration of drug in the brain ISF (by definition unbound)
$C_{u,cell}$	Average concentration of unbound drug in brain cells
$C_{u,plasma}$	Unbound concentration in plasma
$C_{u,ss,brainISF}$	Unbound steady-state concentration in brain ISF
$C_{u,ss,plasma}$	Unbound steady-state concentration in plasma
ECF	Extracellular fluid in the brain (also called ISF, interstitial fluid)
$f_{u,brain}$	Fraction of unbound drug in brain homogenate
$f_{u,brain,corrected}$	Fraction of unbound drug in brain homogenate after correction for pH partitioning based on the pKa(s) of the drug
$f_{u,D}$	Fraction of unbound drug in diluted brain homogenate
$f_{u,plasma}$	Fraction of unbound drug in plasma
GI	Gastrointestinal
ICF	Intracellular fluid in the brain
ISF	Interstitial fluid in the brain (also called ECF, extracellular fluid)
K_i	Inhibition constant

K_{in}	In situ brain perfusion unidirectional transfer constant (a clearance estimate equal to PS or CL_{in}) (µl/min/g_brain)
$K_{p,brain}$	Partition coefficient (ratio) of total brain to total plasma drug concentrations
$K_{p,u,brain}$	Ratio of total brain drug concentration to plasma unbound drug concentration
$K_{p,uu,brain}$	Ratio of brain ISF to plasma unbound drug concentrations
$K_{p,uu,cell}$	Ratio of brain ICF to ISF unbound drug concentrations
$K_{p,uu,CSF}$	Ratio of CSF to plasma unbound drug concentrations
logBB	Logarithm of the ratio of total brain to total plasma drug concentrations (equal to K_p)
MDCK cells	Madin-Darby canine kidney cells
Mdr1	Gene encoding for P-glycoprotein
P_{app}	Unidirectional apparent permeability coefficient measured in the apical-to-basolateral direction (cm/s)
PBS	Phosphate-buffered saline
PET	Positron emission tomography
P-gp	P-glycoprotein
PS	Permeability surface area product (in this context equal to net influx clearance to the brain) (µl/min/g_brain)
V_{blood}	Volume of blood in brain tissue
V_f	Volume of buffer film remaining around the sampled brain slice
V_i	Apparent volume of distribution of a peripheral brain compartment i
V_{ISF}	Physiological (and apparent) volume of ISF
$V_{u,brain}$	Volume of distribution of unbound drug in brain (ml/g_brain)

7.1 Introduction

The delivery of drugs from blood to brain takes place across the brain capillary endothelial cells comprising the blood-brain barrier (BBB). This is depicted in Fig. 7.1 in a classical electron micrograph of a capillary, the extremely thin endothelial cell layer and the brain parenchymal cells. Despite its thinness, the BBB is a very important organ that controls the brain environment in relation to blood, picking up nutrients, discarding waste products, and hindering influx of potentially harmful substances, including many drugs. The large surface area of the BBB and the high rate of blood flow to the brain ensure fast delivery of drugs to the brain (see Chap. 1 and Appendix for anatomical and physiological details of the BBB), but do not always ensure adequate drug concentrations within the brain.

This fact, together with earlier often inadequate methods used for measuring brain drug delivery, has caused problems in central nervous system (CNS) drug
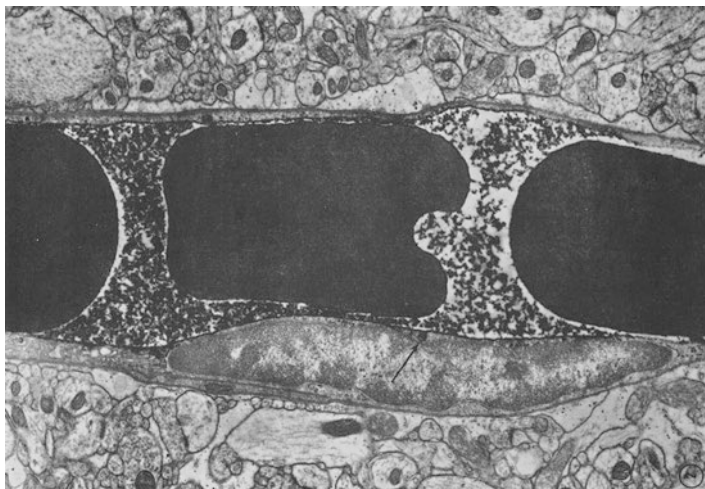
Fig. 7.1 An electron micrograph of a brain capillary with three erythrocytes, endothelial cell walls comprising the BBB, and brain parenchymal cells. The black color indicates intravenously administered peroxidase that does not pass the endothelial cells. The micrograph shows the two membranes of the BBB, the luminal membrane facing the blood and the abluminal membrane facing the brain parenchyma (x20 000). From Reese and Karnovsky with permission from the publisher (Reese and Karnovsky 1967)

discovery and development, due to measuring mainly total drug concentrations in the brain. The methods used in the industry are developing rapidly; these methods are discussed further in other chapters. This chapter focuses on the pharmacokinetic principles of small molecular drug delivery to the brain, on the rate and extent of drug transport as two separate factors governing drug delivery to the brain and on the pharmacokinetic parameters needed to describe this.

Figure 7.2 provides a more schematic drawing of how drugs are distributed across the BBB and into the brain. As depicted, it is only the unbound drug molecules, i.e., those that are not bound to plasma proteins that are able to transverse membranes, in this case the BBB. The rate at which the drug enters the brain interstitial fluid (ISF, also called extracellular fluid (ECF)) depends on the permeability of the BBB to the particular molecule. Together with the passive and active uptake and efflux processes at the BBB, this will determine how much drug enters the brain ISF. The drug molecules will then be further distributed to and equilibrated within the brain cells, specific and nonspecific binding sites, and organelles, depending on the physicochemical interactions between the drug and the tissue.

Drug transport between blood and cerebrospinal fluid (CSF) takes place at the blood-CSF barrier (BCSFB). There is also some exchange between CSF and brain ISF. Transport from CSF to ISF involves passive diffusion, while transport from ISF to CSF involves both passive diffusion and bulk flow of ISF, including possible influence of the "glymphatic" flow (Cserr et al. 1977; Nicholson and Sykova 1998; Abbott et al. 2018; Iliff et al. 2012). See also Chap. 1. The pH of blood is 7.4, while
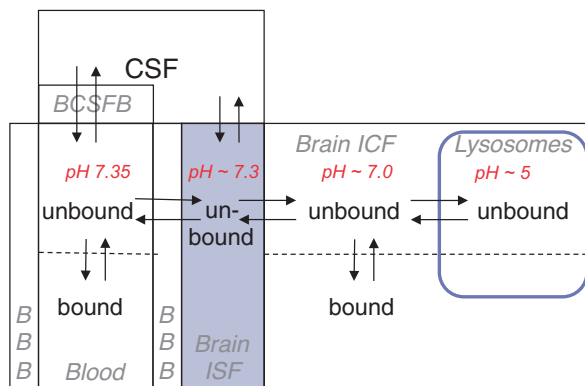
Fig. 7.2 Schematic illustration of drug distribution and equilibration across the BBB and other membranes within the brain parenchyma and unbound drug and drug bound to tissue components. The physiological volumes of the intra-brain compartments are brain interstitial fluid (ISF) 0.2 ml/g_brain and brain intracellular fluid (ICF) 0.8 ml/g_brain, of which the lysosomal compartment is 0.01 ml/g_brain. The figure is adapted from Hammarlund-Udenaes et al. (Hammarlund-Udenaes et al. 2008) with permission from the publisher

that of brain ISF is around 7.3, of the cell cytosol is 7, and in lysosomes is around 5.2. These pH differences influence drug equilibration, with basic drugs accumulating more in low-pH organelles, especially in the lysosomes. By definition, the concentrations in brain ISF are unbound, as are the concentrations in the intracellular fluid (ICF). The extent of nonspecific binding is generally quantitatively much greater than that of specific binding to receptors or other target sites.

It is only the unbound drug that is in contact with receptor or other target sites, and experimental data show that these concentrations are best correlated with clinical effects or side effects in the brain (Hammarlund-Udenaes 2010; Watson et al. 2009; Kalvass et al. 2007b; Large et al. 2009). The site of action of the particular drug will determine whether brain ISF or brain ICF concentration is the more important in relation to the pharmacodynamic measurement. It has been clearly shown for dopamine agonists and other drugs that the unbound drug brain concentrations are much more closely related to receptor occupancy than the total brain concentrations or the concentrations of unbound drug in the blood (Watson et al. 2009; Stevens et al. 2012). This is clearly shown in Fig. 7.3, which depicts the receptor occupancy of several dopamine antagonists in relation to their plasma, total brain, and unbound drug brain concentrations.

The amount of drug to be delivered to the brain to achieve the desired effect is of course always an issue when deciding on the dose to be administered. However, a trade-off between side effects and the desired effects also needs to be taken into consideration. For drugs that are very efficiently effluxed at the BBB, there will be much lower unbound concentrations in brain ISF than in plasma. This is advantageous if peripheral effects and avoidance of CNS side effects are desired, but is less suitable if CNS effects are desired and peripheral side effects are to be avoided.
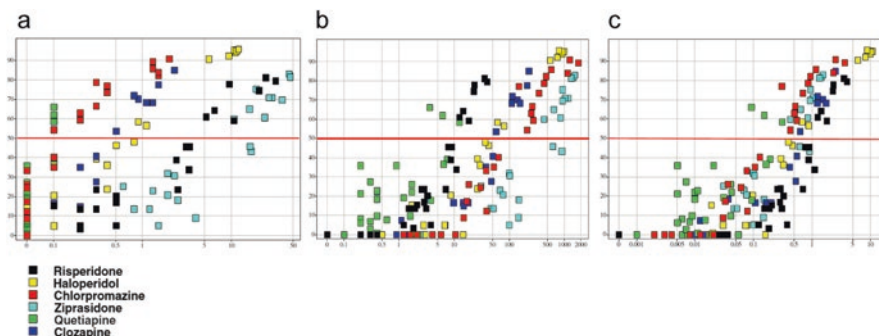
Fig. 7.3 Relationships between receptor occupancy and concentrations of neuroleptics normalized for their in vitro affinity for rat striatal D_2 receptors. a) Total plasma concentrations, b) total brain concentrations, and c) unbound brain concentrations, illustrating the clear advantage of unbound brain concentrations when comparing drugs. Reprinted from Watson et al. 2009 () with permission from the publisher

For measurements based on pharmacokinetic principles, drug delivery can be described by three distinctly different parameters. Two of these are important components of the transport of the drug across the BBB, and the third describes the intra-brain distribution of the drug. The first parameter describes the **rate of drug delivery to the brain** based on the permeability surface area product (PS), which in pharmacokinetic literature is often called the net influx clearance (CL_{in}, µl/min/g_brain). This describes the unidirectional net drug transport from blood to brain. The second parameter is the **extent of delivery**, which can be described either by the total drug concentrations in the brain and plasma or by unbound drug concentrations at steady state. The total drug concentration ratio between the brain and plasma is termed $K_{p,brain}$. Another way of describing the same parameter is logBB, which is also used for computational approaches (Abraham et al. 1995; Norinder and Haeberlein 2002; Young et al. 1988; Norinder et al. 1998; Mensch et al. 2010a; Sun 2004; Shityakov et al. 2013; Muehlbacher et al. 2011; Fan et al. 2010). The unbound drug concentration ratio between brain ISF and plasma is termed $K_{p,uu,brain}$ (Gupta et al. 2006). The relationship between the unbound and total drug concentrations in plasma is described by the fraction of drug that is not bound to plasma proteins, $f_{u,plasma}$. There are two alternative measurements in brain parenchymal tissue that can be used to describe **intra-brain distribution**, the third parameter. This parameter correlates unbound to total drug concentrations in the brain. $f_{u,brain}$ is the fraction of unbound drug in the brain based on brain homogenate measurements (Kalvass and Maurer 2002), and $V_{u,brain}$ is the unbound volume of distribution within the brain in ml/g_brain tissue based on brain slice measurements (; Friden et al. 2007, 2009a; Kakee et al. 1996; Loryan et al. 2013). It should be noted that this volume term is not the same as those determined from in situ brain perfusion or PET studies. In the coming sections, these three parameters will be described in more detail. In vitro and in vivo methods used for determining brain drug delivery are further described in other chapters.

7.2 Historical Aspects on Studying Brain Drug Delivery

Several expressions have been used to describe drug delivery to the brain in the literature: permeation (Tamai and Tsuji 2000; Abbott et al. 2008), brain penetration (Schinkel et al. 1996), extent of brain penetration (Liu et al. 2008), CNS penetration (Summerfield et al. 2006), BBB penetration (Gunn et al. 2012), brain delivery (Pardridge et al. 1992), and CNS distribution (Dai et al. 2005; Kalvass et al. 2007a). The expressions used for the total brain to total plasma concentration ratio also vary: [brain]/[plasma] (Kalvass and Maurer 2002), K_p (a classical expression in pharmacokinetics for the partition coefficient between tissue and plasma (Rowland and Tozer 2011)), $K_{p,brain}$ (Gupta et al. 2006), and B/P (Maurer et al. 2005). Expressions for the brain to blood (or vice versa) unbound drug concentration ratios have been described as $K_{p,uu}$ (Gupta et al. 2006), $K_{p,free}$ (Liu et al. 2005), and [plasma]$_u$/[brain]$_u$ (Kalvass et al. 2007a).

Kalvass and Maurer made a seminal contribution in 2002 by initiating investigation into how to find out whether drugs are actively effluxed at the BBB (Kalvass and Maurer 2002), after P-gp had been found in the BBB (Tsuji et al. 1992; Cordon-Cardo et al. 1989; Thiebaut et al. 1989) and after the development of the P-gp knockout mouse model (Schinkel et al. 1996). They introduced the in vitro brain homogenate binding method in this context and simplified the estimation of extent of drug binding from diluted brain homogenate samples. The ratio of the fraction of unbound drug in plasma to that in the brain ($f_{u,plasma}$/$f_{u,brain}$) was compared with the ratio of total brain to plasma concentrations ($K_{p,brain}$). Kalvass and Maurer concluded that, if the two ratios are the same, the drug will be transported across the BBB mainly by passive means. Efflux was indicated by differences between the ratios, i.e., this was an indirect way of describing BBB transport properties. We know today that the ratio of $f_{u,plasma}$/$f_{u,brain}$ itself as an indication of partitioning between brain and blood is misrepresentative, as the main cause of deviations in $K_{p,brain}$ from this ratio is active transport at the BBB. The authors also compared CSF concentrations to brain and plasma concentrations and found that CSF concentrations over-predicted brain exposure for P-gp substrates.

Maurer et al. continued the work with a comparison of plasma and brain concentrations for 33 compounds (Maurer et al. 2005). Differences in $f_{u,plasma}$/$f_{u,brain}$ within a threefold range were allowed, to cope with experimental errors and differences considered of little consequence for pharmacology or pharmacokinetics. The authors stated that "Because the brain to plasma ratio (K_p) is determined largely by nonspecific binding, efforts to optimize this parameter may actually lead to an unproductive or counterproductive design of drugs that are unnecessarily basic, lipophilic, and simply have a greater degree of nonspecific partitioning into brain tissue" (Maurer et al. 2005). This has proven to be a very relevant statement, which partly explains the poor success rate in developing new drugs for CNS diseases (Kaitin 2008; Kola and Landis 2004). They also surmised that the underprediction of tissue distribution of bases, but not of neutral compounds and acids, based on

$f_{u,brain}$ values could be the result of disruption of the subcellular acidic organelles such as lysosomes during homogenization.

Data from the literature were used by Kalvass and coauthors to compare more drugs, using the correlations developed earlier by Kalvass and Maurer (Kalvass et al. 2007a; Kalvass and Maurer 2002). They commented that $K_{p,brain}$ was still (in 2007) used to optimize brain delivery (values of ≥1 were arbitrarily given an interpretation of good brain delivery and values << 1 of poor brain delivery) and issued another warning that this classification could be misleading, as $K_{p,brain}$ is also influenced by the relative extent of binding to plasma proteins and brain tissue (Kalvass et al. 2007a). A ratio based on plasma to brain concentrations of unbound drug was proposed ([plasma]$_u$/[brain]$_u$), and a log-log graph which plotted the in vivo P-gp efflux ratio vs [plasma]$_u$/[brain]$_u$ was developed. Their conclusions on the BBB transport of the studied drugs were based on the quadrant into which the drug fell. This way of estimating BBB transport is further discussed by Avdeef in his book (Avdeef 2012). Kalvass et al. found indications of active uptake at the BBB and also found that efflux transport mediated by transporters other than P-gp was not able to be accurately predicted by the P-gp efflux ratios in Mdr1a(+/+) and Mdr1a(−/−) mice. For ten of the 34 drugs studied, the extent of efflux in vivo was greater than could be explained by P-gp, and active uptake into the brain was indicated for three drugs. Thus, the in vivo P-gp efflux ratio for knockout and wild-type mice was not sufficient to predict brain delivery, and the [plasma]$_u$/[brain]$_u$ ratio was better predictive than the P-gp efflux ratio alone (Kalvass et al. 2007a). Despite this, most drug companies continue to trust P-gp efflux ratios in vivo or in vitro as the parameter of choice.

Concepts around the BBB transport of drugs were developed further by our group, with the proposal of the term $K_{p,uu}$ by Gupta et al. to succinctly describe the brain ISF to blood concentration ratio for unbound drug (Gupta et al. 2006). Before the publication of this expression in 2006, the efficiency of net active efflux or uptake for individual drugs had been described as the "ratio of unbound brain to unbound blood concentrations" (Bouw et al. 2000, 2001; Xie et al. 2000; ; Tunblad et al. 2003; 2004a, b, 2005 Bostrom et al. 2005). The approach thus separated BBB transport properties from protein binding in plasma and binding to brain constituents, treating the three parameters as independent, individual properties of the drugs. It was indicated that the permeability of the brain to the drug (PS, CL_{in}) and the extent of equilibration across the BBB ($K_{p,uu,brain}$) were not correlated (Hammarlund-Udenaes 2000; Hammarlund-Udenaes et al. 2008; Hammarlund-Udenaes et al. 1997). The brain slice technique was also developed for studies of nonspecific binding to brain tissue in a high-throughput model and was compared with the brain homogenate method (Friden et al. 2007, 2009a, 2011 Loryan et al. 2013).

Doran et al. concluded that most CNS drugs have some degree of P-gp-mediated transport and that this does not hamper their clinical use (Doran et al. 2005). They studied the total brain to plasma, CSF to plasma, and CSF to brain concentration ratios in Mdr1a(+/+) and Mdr1a(−/−) mice without taking into account differences between the drugs in nonspecific binding in the brain. They found that despite being a good P-gp substrate, risperidone has sufficient clinical effect in the CNS because

of its high potency; the question of the correct dose in relation to peripheral side effects is also pertinent here.

At around the same time, Liu and coworkers published on properties that govern the equilibration of drug concentrations between brain and blood (Liu et al. 2005). They concluded that rapid permeation alone does not guarantee rapid equilibration. What is required for rapid equilibration is a combination of rapid permeation and low brain tissue binding. The authors used permeability as a surrogate for efflux clearance, although they are not strictly interchangeable. Nonetheless, the combination of efflux clearance from the brain and the extent of brain binding determines the equilibration time across the BBB (Hammarlund-Udenaes et al. 1997; Liu et al. 2005; Syvanen et al. 2006).

Liu et al. proposed a direct extrapolation of $f_{u,plasma}$ to describe $f_{u,brain}$ as they (Liu et al. 2005) and others (Kalvass and Maurer 2002; Maurer et al. 2005) found a good correlation between the two ($r^2 = 0.69$ (Liu et al. 2005)). Although the use of $f_{u,plasma}$ for $f_{u,brain}$ has not been evaluated any further, its use can be questioned today if a good estimation of $K_{p,uu,brain}$ is the goal. Even a twofold difference between the two will result in a twofold difference in the value of $K_{p,uu,brain}$ and could skew information on the parameter needed for selection of the best drug candidates (see further Sect. 7.3.2.2).

Liu and Chen also discussed the extent and rate of brain penetration by looking at ways to increase the $K_{p,uu,brain}$ by reducing the efflux clearance or increasing the influx clearance (Liu and Chen 2005). In this paper, $K_{p,brain}$ was considered unsuitable for evaluation of the potential success of a candidate as a CNS drug. Liu et al. later proposed strategies for studying transporters at the BBB, including: "1) Drug discovery screens should be used to eliminate good P-gp substrates for CNS targets. Special consideration could be given to moderate P-gp substrates as potential CNS drugs based on a high unmet medical need and the presence of a large safety margin. 2) Selection of P-gp substrates as drug candidates for non-CNS targets can reduce their CNS-mediated side effects" (Liu et al. 2008).

Several articles in the area have also been published by Summerfield and coworkers. In one study, they used Mdr1a/b(+(+) and Mdr1a/b(−/−) mice to investigate total brain to blood ratios ($K_{p,brain}$) in vitro, covering a wide range of physicochemical properties (Summerfield et al. 2006). They also compared $f_{u,brain}$ and $f_{u,blood}$. They concluded that the in vitro estimation of $f_{u,brain}/f_{u,blood}$ overpredicted the K_p observed in vivo because the in vitro ratio assumes that the concentrations in brain and blood are equal, while in reality they are not, because of active transport in the BBB. In their next study, they investigated 50 marketed drugs and compared in situ brain perfusion permeability with in vitro permeability and then correlated these parameters with physicochemical information (Summerfield et al. 2007). In their 2008 publication they studied species differences in plasma and brain binding and found a good correlation in brain binding between rat, pig, and humans, thereby improving the prediction of drug distribution to the brain in humans; they also published a table defining PET and pharmacokinetic expressions (Summerfield et al. 2008). The use of PET and in vitro equilibrium dialysis to assess BBB transport of candidate drugs in CNS drug development was advocated in a later publication (Gunn et al.

2012). An integrated approach involving permeability, active efflux, and brain distribution, and focusing on unbound drug, was proposed by Jeffrey and Summerfield (Jeffrey and Summerfield 2010). In a later paper, they state that "Assessing the equilibration of the unbound drug concentrations across the blood-brain barrier ($K_{p,uu}$) has progressively replaced the partition coefficient based on the ratio of the total concentration in brain tissue to blood (K_p)" (Summerfield et al. 2016).

Hakkarainen et al. compared the in vitro apparent permeability coefficient (P_{app}) from three cell culture systems with in vivo microdialysis measuring $K_{p,uu,brain}$ for nine drugs (Hakkarainen et al. 2010). Unfortunately, the use of an in vitro microdialysis probe recovery method in this otherwise thorough paper potentially affected the accurate measurement of the ISF concentrations and thus the $K_{p,uu,brain}$ values. When the results for two P-gp substrates were omitted, the authors found an extremely good correlation between the permeability of BBMEC cells and the microdialysis results ($r = 0.99$) and noted that the lower the permeability, the lower the $K_{p,uu,brain}$. When the drugs known to be P-gp substrates were included, the relationship became nonsignificant, as would be expected since lower $K_{p,uu,brain}$ values indicate more active efflux and are not correlated with permeability per se, as discussed above and below.

7.3 Parameters Describing Drug Delivery to the Brain

7.3.1 Rate of Brain Drug Delivery

7.3.1.1 What and Why

Permeability as a measurement of drug delivery to the brain has historically been the most common way of optimizing drug delivery to this area. Permeability measurements give an estimate of the unidirectional rate of transport of a drug across the BBB in situ or in a cell model in vitro. Rather than telling us how much drug has equilibrated across the BBB at steady state, these measurements tell us how fast the drug is transported across the BBB into the brain.

Permeability measurements are based on the tradition of studying gastrointestinal (GI) absorption. Physiological differences between the GI tract and the BBB, however, make this concept less translatable. Many articles have compared permeability values from in silico predictions, in vitro cell models, in situ methods, and in vivo methods (Summerfield et al. 2007; Abbott et al. 2008; Bickel 2005; Friden et al. 2009b; Hammarlund-Udenaes et al. 2009; Chen et al. 2011; Avdeef and Sun 2011; Avdeef 2011; Di et al. 2009; 2012; Broccatelli et al. 2012; Fan et al. 2010; Liu et al. 2004; Lanevskij et al. 2013; Mensch et al. 2010a, b; Levin 1980; Garberg et al. 2005; Abbott 2004b). Quite commonly, methods measuring the rate of permeation are compared with those measuring the extent of permeation (Pardridge 2004; Hakkarainen et al. 2010).

7.3.1.2 Methods and Relationships

Permeability is described by the rate of permeation in cm/s, obtained by dividing the PS value estimated from in situ brain perfusion (called K_{in}) by the luminal surface area of the vascular space, estimated to be 150 cm^2/g_brain in vivo in rats (Fenstermacher et al. 1988), or by dividing by the surface area of the cell culture in vitro. The in vitro measurement is called P_{app}, the apparent permeability coefficient. In vitro methods include BBB-specific cell models from different origins, as well as Caco-2 or MDCK cells (please see other chapters in this book).

The in situ brain perfusion method is a very elegant way of rapidly determining permeability in an animal model (Takasato et al. 1984; Smith and Allen 2003; Banks et al. 1997). It can also be performed in genetically modified mice to study the influence of active transporters (Dagenais et al. 2000). Examples of CL_{in} (K_{in}) values from in situ brain perfusion and microdialysis studies are given in Table 7.1. It can clearly be seen, when Mdr1a(+/+) and Mdra1a(−/−) mice are compared, that CL_{in} is decreased in the presence of P-glycoprotein (P-gp). CL_{in} therefore describes the net influx clearance across the BBB. In general, the permeability of the BBB to a drug appears to be less critical to drug delivery than the influence of active efflux transporters. More about the pharmacokinetic aspects and relationships of the transport processes at the BBB can be found in Sect. 7.3.5.

7.3.2 Extent of Brain Drug Delivery

7.3.2.1 What and Why

The extent of drug delivery to the brain is based on steady-state measurements of the ratios of total concentrations in brain and plasma (the partition coefficient $K_{p,brain}$ or logBB), total concentrations in brain and unbound concentrations in plasma ($K_{p,u,brain}$), or unbound concentrations in brain ISF and plasma ($K_{p,uu,brain}$). In comparison to absorption from the GI tract, the amount of drug delivered to the brain can be compared with the bioavailability of drug in the brain, although the determining forces are somewhat different.

The most important advantage of using $K_{p,uu,brain}$ instead of $K_{p,brain}$ lies in its ability to, during the drug discovery process, predict the success of drugs intended for action within the brain or, conversely, for the avoidance of side effects in the brain. $K_{p,uu,brain}$ is the parameter that most closely relates to the drug's pharmacodynamic profile, if the receptors are situated facing the brain ISF. If the relevant receptors are intracellular, further investigations are required (see Sect. 7.3.4 and, in more detail, Chap. 13). The $K_{p,uu,brain}$ value is not influenced by plasma protein binding and brain parenchymal binding that would otherwise confound its interpretation. It gives a concrete value to the net result of passive and active transport across the BBB.

Table 7.1 Examples of in situ/in vivo CL_{in} values obtained by in situ brain perfusion or microdialysis

Drug	CL_{in} (µl/min/g _brain)	CL_{in} in Mdr1a (−/−) mice (µl/min/g_brain)	Species	References
Alfentanil	1940	2290	Mouse	Zhao et al. (2009)
Antipyrine	492	–	Rat	Avdeef and Sun (2011)
Atenolol	1.8	–	Rat	Avdeef and Sun (2011)
Cimetidine	7	11	Mouse	Zhao et al. (2009)
Colchicine	9	19	Mouse	Zhao et al. (2009)
Diazepam	2500	2500	Mouse	Zhao et al. (2009)
DPDPE	0.547	6.36	Mouse	Dagenais et al. (2004)
Fentanyl	1840	2280	Mouse	Dagenais et al. (2004)
Fexofenadine	3	13	Mouse	Zhao et al. (2009)
Imipramine	1860	–	Rat	Avdeef and Sun (2011)
Loperamide	100	1030	Mouse	Dagenais et al. (2004)
Methadone	420	1090	Mouse	Dagenais et al. (2004)
Morphine	10.4	12.9	Mouse	Dagenais et al. (2004)
Morphine	11.4	–	Rat	Bouw et al. (2000) and Tunblad et al. (2004b)
Morphine-3-glucuronide	0.11	–	Rat	Xie et al. (2000)
Morphine-6-glucuronide	1.66	–	Rat	Bouw et al. (2001) and Tunblad et al. (2005)
Oxycodone	1910	–	Rat	Bostrom et al. (2006)
Phenytoin	334	347	Mouse	Zhao et al. (2009)
Quinidine	34	541	Mouse	Zhao et al. (2009)
Ritonavir	23	80	Mouse	Zhao et al. (2009)
Sufentanil	340	295	Mouse	Zhao et al. (2009)
Terfenadine	1740	2020	Mouse	Zhao et al. (2009)
Valproate	243	181	Mouse	Zhao et al. (2009)
Verapamil	315	1370	Mouse	Zhao et al. (2009)

When $K_{p,uu,brain}$ is combined with the target binding properties of the drug, it is possible to estimate the required plasma concentrations, and thus the doses, for pharmacological success. There is no clear cutoff point below which a drug is not suitable for action within the brain, but the lower the $K_{p,uu,brain}$ value, the higher is the dose required to obtain pharmacologically relevant concentrations in the brain given similar potency. The trade-off is more between a dose that can be administered in relation to clinical effect vs side effects and a dose that is economically defendable.

7.3.2.2 Methods and Relationships

The $K_{p,brain}$ ratio can be determined by measuring steady-state drug concentrations or the area under the concentration-time curves in brain tissue, excluding capillary blood concentrations ($AUC_{tot,brain}$), and plasma ($AUC_{tot,plasma}$) after a single dose:

$$K_{p,brain} = \frac{AUC_{tot,brain}}{AUC_{tot,plasma}} \tag{7.1}$$

Measuring the AUC after a single dose is comparable to taking samples of brain and blood at one time point during steady state. The AUCs can then be substituted by the steady-state drug concentrations.

$K_{p,uu,brain}$ can be determined directly from microdialysis samples from brain and plasma sites or by measuring total brain and plasma concentrations at steady state combined with plasma protein binding (giving the fraction of unbound drug in plasma, $f_{u,plasma}$) and brain slice or brain homogenate measurements of nonspecific binding to brain parenchyma (Friden et al. 2007, 2009a, 2010; Loryan et al. 2016):

$$K_{p,uu,brain} = \frac{AUC_{u,brainISF}}{AUC_{u,plasma}} = \frac{AUC_{tot,brain}}{AUC_{tot,plasma} {}^* V_{u,brain} {}^* f_{u,plasma}} \tag{7.2a}$$

Here, $AUC_{u,brainISF}$ describes the concentrations of unbound drug in brain ISF, and $AUC_{u,plasma}$ describes the concentrations of unbound drug in plasma. $V_{u,brain}$ measured with the brain slice method may be replaced by $1/f_{u,brain}$ after correction for pH partitioning if a brain homogenate is used to determine the nonspecific brain binding, as described in Eq. 7.2b.

$$K_{p,uu,brain} = \frac{AUC_{tot,brain} {}^* f_{u,brain,corrected}}{AUC_{tot,plasma} {}^* f_{u,plasma}} \tag{7.2b}$$

Thus, $V_{u,brain}$ is similar but not equal to $1/f_{u,brain}$, which can result in different results if pH partitioning is not compensated for (Friden et al. 2011). More about the similarities and differences between these parameters is given in Sect. 7.3.3 and in Chap. 13. As the combined method involves measuring three individual parameters, the experimental error in each of them will affect the $K_{p,uu,brain}$ estimate (Kalvass et al. 2007a). Here, the uncertainty propagation method can be used (Loryan et al. 2017; Yusof et al. 2019).

The concentration of drug in brain ISF is determined by diffusion, transport, metabolism, and binding processes, as described in Fig. 7.1. The differential equations describing the equilibration across the BBB between unbound drug in plasma and the brain ISF compartment are:

$$\frac{V_{ISF} {}^* dC_{u,brainISF}}{dt} = CL_{in} {}^* C_{u,plasma} - \left(CL_{out} + CL_i \right) * C_{u,brainISF} + CL_i {}^* C_i \tag{7.3}$$

$$\frac{V_i^* dC_i}{dt} = CL_i^* \left(C_{u,brainISF} - C_i \right) \tag{7.4}$$

V_{ISF} describes both the physiological volume of the ISF and the apparent volume of distribution in the ISF, as it is assumed that there is no binding in this compartment. CL_{in} and CL_{out} describe the net influx and efflux clearance across the BBB. CL_{in} is equivalent to PS. V_i and C_i are the apparent volume of and drug concentration in a possible deeper brain compartment i, and CL_i is the intercompartmental clearance between this compartment and the ISF. The plasma unbound drug concentration ($C_{u,plasma}$) is the driving force for the brain concentrations. Further equations necessary to describe the plasma concentration-time profile are beyond the scope of this chapter.

At steady state, there is no change in concentration in brain ISF, $dC_{u,brainISF}/dt = 0$, and the drug concentrations in plasma ($C_{u,ss,plasma}$) and brain ($C_{u,ss,brainISF}$) are in equilibrium. If $C_{u,brainISF} = C_i$, which can be assumed since C_i describes a hypothetical compartment, the relationship in Eq. 7.3 becomes:

$$CL_{in}^* C_{u,ss,plasma} = CL_{out}^* C_{u,ss,brainISF} \tag{7.5}$$

As $K_{p,uu,brain}$ is a steady-state parameter, it is not influenced by the further partitioning of the drug into brain cells:

$$K_{p,uu,brain} = \frac{C_{u,ss,brainISF}}{C_{u,ss,plasma}} = \frac{CL_{in}}{CL_{out}} \tag{7.6}$$

It can be seen in Eq. 7.6 that $K_{p,uu,brain}$ is determined by the relative size of the net influx and efflux clearances. This means that influx and efflux clearances can both be small and large and still result in the same $K_{p,uu,brain}$. This explains why the permeability per se is not the most important parameter for estimating the extent of drug delivery to the brain. While rapid delivery to and elimination from the brain is clinically important for, for example, anesthetic drugs, the steady-state concentration in the brain is more important than the rate of delivery to the brain when a drug is to be administered repeatedly over time. The range of CL_{in} values within which brain delivery is still sufficient can, therefore, be quite wide. This is exemplified in Table 7.1 by the good clinical effects of morphine despite its low permeability clearance vs the lack of clinical effect of loperamide despite its higher permeability clearance. This phenomenon is also illustrated in Fig. 7.4.

Equation 7.6 can be further developed to include the different processes governing the uptake and elimination of drug from brain ISF:

$$K_{p,uu,brain} = \frac{CL_{in}}{CL_{out}} = \frac{CL_{passive} + CL_{act\_uptake} - CL_{act\_efflux}}{CL_{passive} - CL_{act\_uptake} + CL_{act\_efflux} + CL_{bulk\_flow} + CL_{metabolism}} \tag{7.7}$$
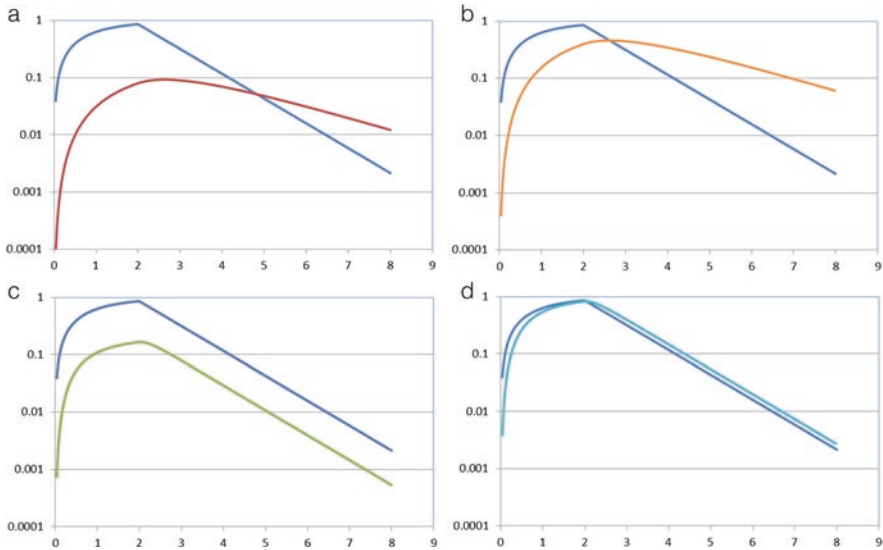
Fig. 7.4 Illustration of the absolute values of CL_{in} and CL_{out} and their relationships with the resulting brain concentration-time profile of unbound drug with time on the x-axis and concentration on the y-axis. The blue line, similar in all parts of the figure, describes the unbound drug concentration in blood after a short intravenous infusion of a fictive drug. The other lines describe the brain unbound drug concentrations. The relative values of CL_{in} and CL_{out} are in a) $CL_{in} = 1$, $CL_{out} = 5$ giving a $K_{p,uu,brain}$ of 0.2; b) $CL_{in} = 5$ and $CL_{out} = 5$, giving a $K_{p,uu,brain}$ of 1.0; c) $CL_{in} = 10$ and $CL_{out} = 50$ giving a $K_{p,uu,brain}$ of 0.2; and d) $CL_{in} = 50$ and $CL_{out} = 50$, giving a $K_{p,uu,brain}$ of 1.0. In a) and b), CL_{out} values together with the size of $V_{u,brain}$ (the same in all simulations) result in a longer half-life for the drug in the brain than in blood. In c) and d), the half-life in the brain follows that in blood because of the more rapid processes in the brain than in blood. A comparison of (**a**) and (**c**), (**b**) and (**d**), respectively, shows that the $K_{p,uu,brain}$ is the same, independent of a tenfold difference in CL_{in} and independent of differences in half-lives in the brain

$CL_{passive}$ is the passive diffusional clearance across the BBB, which is assumed to be equal in both directions. CL_{act_efflux} describes the active efflux transport back across the BBB to the plasma (Syvanen et al. 2006). CL_{act_uptake} describes the active uptake transport across the BBB into the brain. Both active transport parameters can include one or several transporter functions and can, if of interest, be further divided into the individual processes. CL_{bulk_flow} is the bulk flow of ISF from brain to CSF, reported to be 0.1–0.3 µl/min/g_brain (Cserr et al. 1977; Rosenberg et al. 1980; Abbott 2004a). $CL_{metabolism}$ describes the elimination of a drug through metabolism within the brain.

Equation 7.7 assumes that $CL_{passive}$ is the same, independent of direction of transport across the BBB. In reality, this may not be correct for the two membranes of the BBB (luminal vs abluminal), as a result of different fluid flow rates and diffusion properties. The equation suggests that active efflux of a drug will reduce CL_{in} and that active uptake will reduce CL_{out}. An experimental illustration of this is provided by the distinct effect of P-gp on CL_{in} that was found by Dagenais et al. (Dagenais

et al. 2004). They used in situ brain perfusion methodology in Mdr1a(+/+) and Mdr1a (−/−) mice. The PS of loperamide increased tenfold from 100 μl/min/g_brain in Mdr1a(+/+) mice to 1030 μl/min/g_brain in Mdr1a (−/−) mice (Table 7.1). It should also be borne in mind that CL_{in} and CL_{out} are the net clearances across both the luminal and abluminal membranes of the brain endothelial cells when, in reality, transporters are usually situated in either the apical or basolateral membrane and are rarely situated in both membranes.

If the only method of transport is passive, or if the influx and elimination processes are of the same magnitude, the unbound concentrations in the brain will equal those in plasma when equilibrium is reached between the two sites. $K_{p,uu,brain}$ will be smaller than unity if efflux dominates the transport process (Gupta et al. 2006; Hammarlund-Udenaes et al. 2008) and greater than unity if active uptake dominates (Bostrom et al. 2006; Hammarlund-Udenaes et al. 2008; Sadiq et al. 2011; Kurosawa et al. 2017). The relationships and their interpretation are further described in Table 7.2.

Most drugs seem to be effluxed at the BBB. This can be seen in Fig. 7.5, which provides the $K_{p,uu,brain}$ values for a selection of drugs that are acids, bases, neutrals, and zwitterions (Friden et al. 2009b).

7.3.3 Intra-Brain Distribution

7.3.3.1 What and Why

Estimation of the extent of nonspecific binding of a drug to brain tissue is necessary in order to relate the total brain concentrations, which are easily measured, to the unbound drug concentrations, which are more difficult to measure but more valuable for optimizing drug treatment. This is an intra-brain measurement and is not related to BBB function.

7.3.3.2 Methods and Relationships

The three methods by which intra-brain distribution can be estimated include microdialysis in the brain in conjunction with a brain sample to provide total brain concentrations at steady state (Wang and Welty 1996; Hammarlund-Udenaes 2013), the brain homogenate method (Kalvass and Maurer 2002; Mano et al. 2002), and the brain slice method (Kakee et al. 1996; Friden et al. 2009a, 2010; Loryan et al. 2013). The microdialysis and brain slice methods result in an estimate of $V_{u,brain}$ in ml/g_brain tissue, while the brain homogenate method results in an estimate of $f_{u,brain}$.

Table 7.2 Relationship between the rate and the extent of equilibration across the BBB. More than one transporter may be acting on the drug and transport can be in either direction. Further examples from a combination of iv infusion, brain slice, and plasma protein binding measurements can be found in Fridén et al. (Friden et al. 2009b)

Parameter value	Relationship	Interpretation	In vivo examples
$K_{p,uu} \approx 1$	$CL_{in} \approx CL_{out}$	Net influx and efflux clearances are similar either because the drug is only passively transported across the BBB or because the active influx and efflux rates are similar. Note that the absolute sizes of the clearances are not important, only the relationship between the two.	Codeine (Xie and Hammarlund-Udenaes 1998) Diazepam (Dubey et al. 1989) Olanzapine, haloperidol (Loryan et al. 2016)
$K_{p,uu} < 1$	$CL_{in} < CL_{out}$	Elimination processes from the brain are more efficient than influx processes. This may be because of more active efflux transport at the BBB, metabolism within the brain parenchyma, or bulk flow (the latter requires clearances to be quite low, as bulk flow is 0.1–0.3 µl/min/g_brain).	Morphine (Bouw et al. 2000; Tunblad et al. 2003; Bostrom et al. 2008) Risperidone and paliperidone (Doran et al. 2012; Liu et al. 2009; Loryan et al. 2016) Ofloxacin, perfloxacin (Ooie et al. 1997) 6-Mercaptopurine, probenecid (Deguchi et al. 2000) Atenolol, methotrexate, paclitaxel (Friden et al. 2009b; Hu et al. 2017; Chen et al. 2017; Westerhout et al. 2014) Quinidine, indinavir, dexamethasone (Uchida et al. 2011a) Quinidine (Westerhout et al. 2013)
$K_{p,uu} > 1$	$CL_{in} > CL_{out}$	Influx processes across the BBB are quantitatively more efficient than efflux/metabolism/bulk flow processes. This can only be accomplished if the drug is actively transported from blood to brain.	Oxycodone (Bostrom et al. 2006) Diphenhydramine (Sadiq et al. 2011) Nicotine (Tega et al. 2013) Varenicline (Kurosawa et al. 2017)

Microdialysis

Microdialysis can be used to determine both $K_{p,uu,brain}$ and $V_{u,brain}$. In order to calculate $V_{u,brain}$, it is necessary to measure total brain concentrations at steady state at the same time as obtaining the concentration of unbound drug in brain ISF by microdialysis.
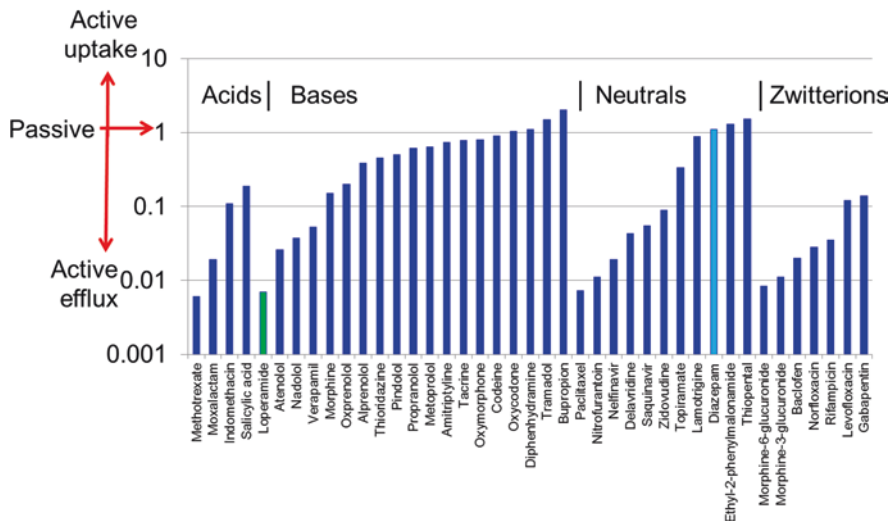
Fig. 7.5 $K_{p,uu,brain}$ values from a combined study of $K_{p,brain}$, $f_{u,plasma}$, and $V_{u,brain}$ in rats. $K_{p,uu,brain}$, to the extent that it can be extrapolated to humans, indicates the clinical usefulness of the drug for action in the brain. The brain ISF concentrations are similar ($K_{p,uu,brain} \approx 1$), lower ($K_{p,uu,brain} < 1$) or higher ($K_{p,uu,brain} > 1$) than the unbound concentrations in plasma. Data from Fridén et al. (Friden et al. 2009b)

The expression $V_{u,brain}$ was introduced by Wang and Welty in their microdialysis study of gabapentin influx and efflux across the BBB (Wang and Welty 1996). The paper was seminal for improving understanding of how the BBB transport of drugs can be evaluated (Hammarlund-Udenaes et al. 2008). $V_{u,brain}$ can be described by Eq. 7.8:

$$V_{u,brain} = \frac{A_{tot,brain\_incl\_blood} - V_{blood}{}^{*}C_{tot,blood}}{C_{u,brainISF}} \tag{7.8}$$

where $A_{tot,brain\_incl\_blood}$ is the amount of drug present per g brain, obtained from chemical analysis of the brain tissue sample. It is then necessary to subtract the amount of drug in the brain capillaries in order to obtain the amount present in the brain tissue itself. V_{blood} is the physiological volume of blood present in the brain tissue sample, and $C_{tot,blood}$ is the total concentration of the drug in the blood. The volume used here is critical for correct estimation of $V_{u,brain}$ (Friden et al. 2010).

Brain Homogenate

The brain homogenate method results in an estimate of $f_{u,brain}$. In short, this method uses fresh or frozen brain homogenate that is diluted with phosphate-buffered saline (PBS) and equilibrated across a dialysis membrane. The method is described in

detail in Chap. 13. Samples of buffer and homogenate are analyzed, and the fraction of unbound drug in the original sample is calculated using Eq. 7.9 to compensate for the dilution:

$$Undiluted\_f_{u,brain} = \frac{1/D}{\left(\left(1/f_{u,D}\right)-1\right)+1/D} \tag{7.9}$$

D is the dilution factor for the brain tissue sample, and $f_{u,D}$ is the fraction of unbound drug in the diluted brain homogenate sample.

There are several advantages associated with the brain homogenate method: It is easy to carry out, using the same equipment as that used for plasma protein binding, high-throughput methodology can be used, and the process can be based on frozen tissue. However, it should be borne in mind that homogenizing the sample can expose sites that normally do not bind the drug in vivo (Liu and Chen 2005). Furthermore, membrane structures are destroyed by homogenization. This excludes the measurement of the influence of possible transport processes and pH differences between the brain parenchymal cells and organelles.

The brain homogenate method was used by Di et al. to compare $f_{u,brain}$ values between species, with subsequent important potential for using animal brain homogenates to estimate the nonspecific binding of drugs in human brain (Di et al. 2011). Summerfield had earlier studied species differences between rat, pig, and humans regarding binding to brain tissue (Summerfield et al. 2008). Human brain regional differences in binding including the influence of disease, in comparison to binding in the rat, were studied by Gustafsson et al. highlighting the need of case-by-case evaluation of regional brain binding in translational CNS research (Gustafsson et al. 2019).

Brain Slice

The brain slice method results in an estimate of $V_{u,brain}$ in ml/g_brain tissue. This method, which provides information that is relevant for issues such as nonspecific binding of drug to tissues, lysosomal trapping, and active uptake of drug into cells, is described in detail in Chap. 13. The brain slice method has been optimized for high-throughput of drugs, using cassettes of five to ten drugs that can be studied simultaneously, although it is important that the total combined concentration of drugs in buffer in the cassette does not exceed 1 μM (Friden et al. 2007, 2009a Loryan et al. 2013).

$V_{u,brain}$ is obtained by dividing the total brain concentration found in the slices by the buffer concentration, which is assumed to describe the ISF unbound concentration. Equation 7.10 is adapted from Eq. 7.8 to the in vitro situation:

$$V_{u,brain} = \frac{A_{slice} - V_f^* C_{buffer}}{C_{buffer}^* \left(1 - V_f\right)} \qquad (7.10)$$

A_{slice} is the amount of drug per gram of slice and C_{buffer} is the concentration of drug in the buffer. V_f is the volume of buffer film that remains around the sampled slice due to incomplete absorption of buffer by the filter paper. Fridén et al. confirmed the value of V_f as 0.094 ml/g_slice (Friden et al. 2009a), in agreement with the original observation by Kakee et al. (Kakee et al. 1996).

7.3.3.3 Interpretations and Caveats

Relevant physiological volumes in brain tissue include the volume of brain ISF at 0.2 ml/g_brain (Nicholson and Phillips 1981; Nicholson and Sykova 1998) and the volume of total brain water at 0.8 ml/g_brain (Reinoso et al. 1997). Thus, drugs with values of $V_{u,brain}$ lower than 0.8 ml/g_brain are predominantly distributed outside the brain cells, with minimal binding to proteins or membranes (e.g., moxalactam, which has a $V_{u,brain}$ of 0.46 ml/g_brain (Friden et al. 2010)). As the values for $V_{u,brain}$ increase further above 0.8 ml/g_brain, intracellular distribution and binding to proteins or membranes also increase (e.g., loperamide, which has a $V_{u,brain}$ of 370 ml/g_ brain (Friden et al. 2010)). $V_{u,brain}$ varies between 0.2 and 3000 ml/g_brain for the drugs studied to date. Table 7.3 provides examples of known $V_{u,brain}$ values and the interpretations that can be made based on this information; currently, the highest value is for thioridazine (Friden et al. 2009b).

When using $V_{u,brain}$ to determine $K_{p,uu,brain}$ (Eq. 7.2a), Fridén et al. indicated that the value of V_{blood} from the literature (Eq. 7.8) may be too high (Friden et al. 2010). This appeared especially true for drugs with low $K_{p,brain}$ values. A low $K_{p,brain}$ can be the result of either very efficient efflux at the BBB or a level of plasma protein binding that greatly exceeds the nonspecific binding of the drug in the brain. The latter situation causes a problem when the value for V_{blood} used in Eq. 7.8 is too high. An improved method was developed for this estimation (Friden et al. 2010). It should be noted that the remaining brain vascular space can vary with the method used to sacrifice the animal.

7.3.4 Intracellular Drug Distribution

The intracellular concentrations of drugs cannot be measured directly. However, information on the intracellular distribution of the drug can be obtained by combining brain slice and homogenate data (Friden et al. 2007, 2009a, 2011; Loryan et al. 2014). $K_{p,uu,cell}$ describes the steady-state ratio of intracellular to brain ISF concentrations of unbound drug, assuming an average concentration ratio for all cell types within the brain. In the drug discovery process, this will extend the available

Table 7.3 Interpretation of $V_{u,brain}$ information. For practical purposes, the value of 0.8 ml/g_brain can be approximated to 1 ml/g_brain. The values were obtained using the brain slice method in rats; for further descriptions, see Friden et al. (Friden et al. 2009b) and Loryan et al. (Loryan et al. 2013)

Parameter value	Interpretation	Examples (ml/g_brain)
$V_{u,brain} < 0.8$ ml/g_brain	Restricted distribution of the drug to the interstitial fluid. Probably very low entrance into cells and very little binding to proteins or membranes.	Morphine-3-glucuronide (0.7) Moxalactam (0.6)
$V_{u,brain} \approx 0.8$ ml/g_brain	Free distribution of the drug in ISF and intracellular fluid and/or slight binding to proteins or membranes.	Salicylic acid (1.0) Zidovudine (1.1)
$V_{u,brain} > 0.8$ ml/g_brain	Binding to proteins or membranes or distribution to subcellular organelles such as lysosomes. The higher the value, the more drug is bound or distributed.	Amitriptyline (310) Atenolol (2.5) Diazepam (20, 17.8) Digoxin (33.1) Gabapentin (4.6) Indomethacin (14) Levofloxacin (1.7) Loperamide (370) Nelfinavir (860) Oxycodone (4.2) Paclitaxel (769) Paroxetine (714) Thioridazine (3333, 2650) Verapamil (54, 47)

information about the distribution of new chemical entities and will help in selecting optimal drug candidates. It is important to measure $K_{p,uu,cell}$ and subsequently estimate the average concentration of unbound drug in brain cells ($C_{u,cell}$), in relation to the pharmacodynamic measurements when the drug has an intracellular site of action or when information about possible active transport processes at the ISF-cellular interface is required. This is also relevant, going even one step further into lysosomal distribution, when predicting and understanding possible side effects due to lysosomal accumulation (Loryan et al. 2017).

$K_{p,uu,cell}$ is calculated as:

$$K_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,brainISF}} = V_{u,brain} {}^* f_{u,brain} \tag{7.11}$$

$V_{u,brain}$ is determined from brain slice experiments and $f_{u,brain}$ is determined from equilibrium dialysis of brain homogenates. The details of how to estimate $K_{p,uu,cell}$ and the further division of this parameter into cytosolic and lysosomal components are further described in Chap. 13. Maurer et al. have mentioned lysosomal accumulation as a possible reason for differences in the distribution of acidic, neutral, and basic drugs between homogenates and in vivo measurements in tissues other than the brain (Maurer et al. 2005). This appears also to be important in brain tissue when

comparing brain slice data with data from brain homogenates (Friden et al. 2011; Loryan et al. 2014).

7.3.5 Combining Rate, Extent, and Intra-Brain Drug Distribution in Brain Pharmacokinetics

It will be obvious by now that the three main properties of brain drug delivery, CL_{in}, $K_{p,uu,brain}$, and $V_{u,brain}$, describe three individual properties of a drug. Figure 7.6 provides the $V_{u,brain}$ and $K_{p,uu,brain}$ values for 41 drugs (Friden et al. 2009b).

It can be seen from the figure that these two properties are not correlated. Two examples in the figure highlight this: loperamide and diazepam. The very low $K_{p,uu,brain}$ of loperamide (0.007) indicates that only 0.7% of the concentration of unbound loperamide in plasma will be present in brain ISF and thus that the efflux of loperamide at the BBB is very efficient. At the same time, loperamide has a high affinity to brain tissue, with a $V_{u,brain}$ of 370 ml/g_brain. The transport of diazepam at the BBB, on the other hand, is mainly passive, with a $K_{p,uu,brain}$ close to 1 and a lower affinity, with a $V_{u,brain}$ of 12 ml/g_brain. Similarly, the permeability clearance has little in common with the size of $K_{p,uu,brain}$. As discussed earlier (Eq. 7.6), the
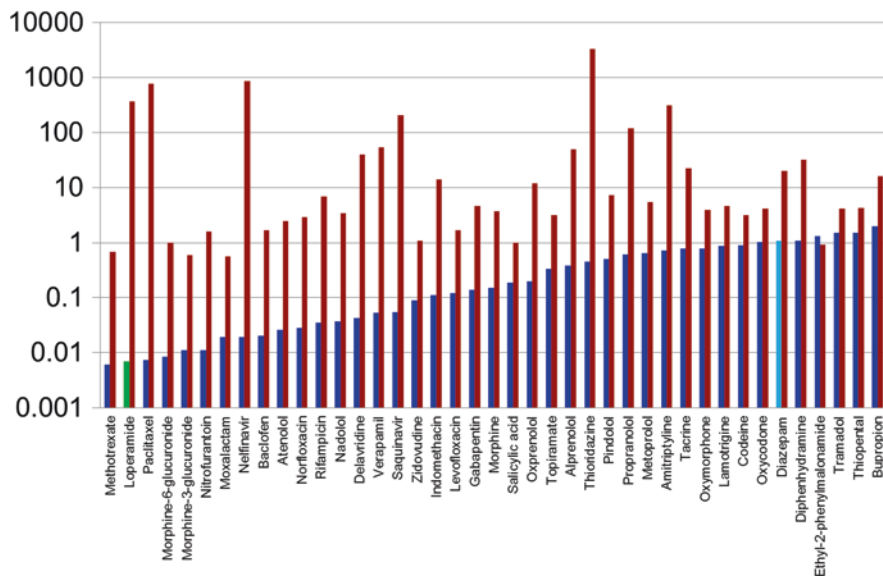

Fig. 7.6 Connections between nonspecific binding in the brain, as shown by $V_{u,brain}$ values (ml/g_brain) and $K_{p,uu,brain}$ ratios for 41 drugs. The scale on the logarithmic y-axis shows the experimentally obtained values for $K_{p,uu,brain}$ and $V_{u,brain}$. The drugs are sorted according to their $K_{p,uu,brain}$ value from smallest to largest. The individual $V_{u,brain}$ values are plotted alongside the $K_{p,uu,brain}$ values and show that there is very little correlation between the two parameters. Data are from Fridén et al. (Friden et al. 2009b)

influx and efflux clearances can both be small and large but can still result in the same $K_{p,uu,brain}$.

The time for drug concentrations to reach equilibrium between brain and blood, on the other hand, is determined by the efflux clearance and the extent of intra-brain binding ($V_{u,brain}$), giving rise to an intrinsic half-life in the brain, which can be shorter or longer than that in plasma. If the plasma half-life is longer than the intrinsic half-life, it will also determine the half-life in brain, which will be equal to that in plasma, and the intrinsic half-life will not be possible to observe. Thus, the unbound drug concentration in plasma is the driving force for the half-life in the brain, and the pharmacokinetic profile in plasma is therefore an important determinant of the concentration-time profile in the brain. Only when elimination of the drug is slower from the brain than from plasma will the intrinsic half-life in the brain be observable. Thus, the plasma concentration-time profile is important for the resulting pharmacodynamics in the brain, be it effects or side effects.

The determinants of the concentration-time profile of a drug in the brain are comparable to the parameters determining the pharmacokinetics in plasma: The plasma concentration-time profile is similarly determined by the absorption and elimination rates and the extent of binding to tissues. The relative unbound concentrations in brain and plasma are determined by the transport process that dominates the movement of the drug at the BBB. This may either be active efflux, active influx, or passive transport as discussed earlier. CL_{in} therefore only influences the brain concentrations (cf bioavailability) in relation to the efflux clearance, but will not influence the concentration-time profile, including the time to reach equilibrium, a fact that may be hard to grasp.

Active efflux of a drug will not only decrease CL_{in} but will also increase CL_{out}, as described in Eq. 7.7, thus increasing the rate of the equilibration processes across the BBB, although this depends on how the efflux transporter functions. If it only hinders influx (the so-called vacuum cleaner model), the efflux from the brain parenchyma will not be influenced, and the active process will not influence the brain elimination half-life (Syvanen et al. 2006). It is, however, more likely that the transporter will both hinder influx and increase efflux (e.g., P-gp). In this case, the part that increases efflux will subsequently affect the elimination process and therefore the time to reach equilibrium across the BBB, while the part that hinders influx will not affect the elimination process and therefore neither the time to equilibrium.

Equilibrium across the BBB is thus reached more quickly for strong P-gp substrates than for drugs that are weaker substrates or that are only passively transported, but otherwise have similar properties. Active efflux also has an important influence on the time aspects of equilibration across the BBB in the studies comparing drug uptake into the brains of Mdr1a/b($-/-$) and Mdr1a/b($+/+$) mice. Equilibration is expected to take longer in Mdr1a/b ($-/-$) mice. When sampling at a specific time after a single dose, this can influence the difference between the two groups of mice. Possible differences in equilibration time therefore need to be taken into consideration.

Padowski and Pollack have discussed the theoretical effects of P-gp on the time to equilibrium across the BBB (Padowski and Pollack 2011), and the theoretical

consequences of active uptake and efflux have also been discussed by several authors (Golden and Pollack 1998; Hammarlund-Udenaes et al. 1997; Syvanen et al. 2006). Liu and Chen have suggested that the parameters determining the half-life of equilibration are the permeability of the BBB to the respective drug and the extent of binding in the brain (Liu and Chen 2005). As explained in this chapter, they are more clearly described as the efflux permeability and the extent of binding in the brain. The slower of the two half-lives in the plasma and brain will determine the observed half-life in the brain.

Cooperation between P-gp and breast cancer resistance protein (BCRP) in increasing the efficiency of the efflux process at the BBB has been clearly described by Kusuhara and Sugiyama (Kusuhara and Sugiyama 2009). The presence and contributions of other, including as yet unknown, transporters should also be included in speculations about the fate of drugs at the BBB (Hammarlund-Udenaes et al. 2008; Kalvass et al. 2007a; Agarwal et al. 2012).

As stated earlier, measurement of unbound drug concentrations in plasma is not enough to determine the unbound concentrations in the brain. Binding to brain parenchymal tissue is too different from binding to plasma proteins to allow prediction of one from the other. The presence of active transport at the BBB does not allow the ratio of the fraction of unbound drug in plasma to that in brain ($f_{u,plasma}$/$f_{u,brain}$) to be used to predict brain penetration, as discussed in Sect. 7.2.

7.4 CSF Pharmacokinetics vs Brain ISF Pharmacokinetics

The CSF is an accessible sampling site for measuring human brain concentrations of unbound drug, given that CSF concentrations follow brain concentrations. However, the role of the CSF as an alternative site for measuring unbound brain concentrations is still under discussion and has not been well established. De Lange and Danhof proposed that the CSF may be of limited value in the prediction of unbound brain concentrations (de Lange and Danhof 2002). There are both similarities and differences in drug concentrations between brain ISF and CSF. The BCSFB, situated between the epithelial cells of the choroid plexus, is different from the BBB as a transport site for drugs, and the cells have different origins (epithelial vs endothelial), which could influence transporter expression (Fig. 7.2). The relevant question for drug discovery is whether the transporter functions in the BBB are similar enough to those in the BCSFB to allow the extrapolation of CSF data to obtain data on the exposure of the brain to unbound drug.

While CSF sampling could be useful in the selection of drug candidates for entry into development programs, Lin cautions that CSF concentrations could differ from brain unbound drug concentrations (Lin 2008). Fridén et al. demonstrated the correlations between rat CSF and rat brain ISF concentrations for 41 compounds (Friden et al. 2009b). In this study, 33 of the $K_{p,uu,brain}$ values were within a ± threefold range of the $K_{p,uu,CSF}$ values, which is considered quite good ($r^2 = 0.80$). However, Fig. 7.7a shows that the regression line deviates from the line of identity for these
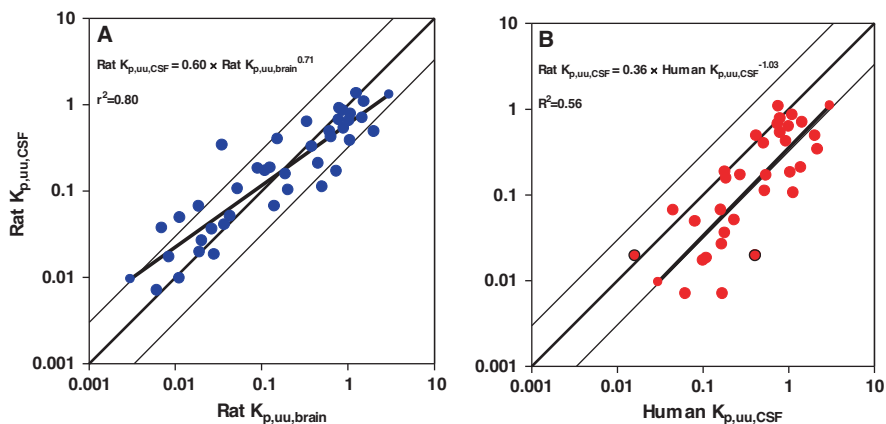
Fig. 7.7 (**a**) Correlations between rat $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ for 41 drugs. The middle diagonal line is the line of identity. The two parallel lines show a threefold difference in range from the line of identity. (**b**) Correlations between $K_{p,uu,CSF}$ in humans (x-axis, data from Shen et al. (Shen et al. 2004)) and $K_{p,uu,CSF}$ in rats (y-axis). Although there is a good correlation between the species, there is a threefold deviation from the line of identity. Reprinted with permission from Fridén et al. (Friden et al. 2009b). Copyright 2009 American Chemical Society

compounds. CSF concentrations were lower than the unbound brain concentrations at high $K_{p,uu,brain}$ values and higher at low $K_{p,uu,brain}$ values. This confirms earlier work by Kalvass and Maurer, who found that unbound brain concentrations were over-predicted by CSF concentrations for drugs with low $K_{p,uu,brain}$ values (Kalvass and Maurer 2002). While the results from Fridén et al. support the use of $K_{p,uu,CSF}$ for comparisons of brain exposure between drugs (Friden et al. 2009b), it should be borne in mind that other drugs could behave differently and that individual drug concentrations could deviate from the predicted value quite extensively.

Differences in the location and expression of P-gp between the BBB and the BCSFB could explain the concentration differences at low $K_{p,uu,brain}$ values. P-gp and BCRP are located in the luminal membranes of the endothelial cells in the BBB. According to an early report, P-gp was thought to be located in the apical membrane of the epithelial cells of the choroid plexus, which would result in substrates being transported toward the CSF (Rao et al. 1999). This has, however, been questioned (Sun et al. 2003). It seems unlikely that P-gp would transport substrates into the CSF in the epithelial cells of the BCSFB and in the opposite direction, into the blood, at the BBB. If this was the case, the CSF would be an even less suitable site of measurement for estimating brain ISF concentrations. Although studies have shown less efficient P-gp functioning at the BCSFB than at the BBB, the findings do not actually support the transport of drugs toward the CSF . The reason for the differences in P-gp function may have been found by Gazzin et al. who measured the relative content of P-gp and Mrp1 protein in rat and human brain capillaries and choroid plexus (Gazzin et al. 2008). They showed that the P-gp content in rat choroid plexus homogenates was only 0.5% of that in brain endothelial cells, while the

opposite trend was seen with Mrp1 – the microvessel content was only 4% of that in the choroid plexus. Human data showed a similar picture. Thus, although it is present at the BCSFB, P-gp seems to have a significantly smaller role than at the BBB because of its lower expression.

The correlation between human and rat $K_{p,uu,CSF}$ is unexpectedly good; however, the threefold deviation from the line of identity, with higher CSF to plasma concentration ratios in humans than in rats (Fig. 7.7b), is an issue not yet explained (Friden et al. 2009b; Shen et al. 2004).

Issues on differences in time between dosage and sampling, and the sites of sampling, in humans vs rodents should also be taken into consideration when studying the use of CSF sampling to estimate drug distribution to the brain. The timing aspects of CSF concentration-time profiles vs brain ISF profiles have been studied by Westerhout et al. using a multiple microdialysis probe approach in rats (Westerhout et al. 2012). It takes only slightly longer to reach similar concentrations of acetaminophen in rat CSF from the cisterna magna and 3rd/fourth ventricles than in brain ISF, although the difference is extended for CSF from the subarachnoid space furthest away from the brain ISF, which is of relevance when sampling CSF in humans. Westerhout et al. developed a physiological pharmacokinetic model for multiple brain compartments, based on these rat data. After translation of the model by changing the physiological parameters to those in humans, they were able to successfully predict lumbar CSF data on acetaminophen comparable to those available from humans. The model also predicted human ISF concentration-time profiles (Westerhout et al. 2012), further developed into a generic model for nine drugs (Yamamoto et al. 2017).

In summary, it appears that CSF is an adequate sampling site for obtaining a preliminary understanding of unbound brain concentrations, provided to be at steady state, with the caveat of taking into account deviations at low and high $K_{p,uu,brain}$ values. The results support the use of $K_{p,uu,CSF}$ for reasonable comparisons of brain exposure to drugs. However, it should be borne in mind that individual drugs could deviate quite extensively from the general correlation.

7.5   Drug Interactions at the BBB

Because transporters play such an important role at the BBB in controlling the traffic of drug molecules into and out of the brain, they may also be targets of clinically significant drug interactions, however rather unlikely (Kalvass et al. 2013). Unfortunately, interaction studies at the BBB in humans are few (Bauer et al. 2012, 2015; Matsuda et al. 2017). Cyclosporin is the most potent P-gp inhibitor on the market, doubling the brain concentrations of verapamil and loperamide (Sasongko et al. 2005; Hsiao and Unadkat 2012). Quinidine also inhibits P-gp in humans, causing a 20% reduction in the response to CO_2 (opiate-induced respiratory depression) when administered with loperamide (Sadeque et al. 2000).

The $K_{p,uu,brain}$ value of a drug can give information on its interaction potential at the BBB (Hammarlund-Udenaes et al. 2008). For a $K_{p,uu,brain}$ close to unity, the interaction potential is likely to be very low, given that the drug is mainly passively transported. The lower the $K_{p,uu,brain}$, the higher the theoretical possibility of an interaction with other drugs, depending on whether the low $K_{p,uu,brain}$ was caused by efflux via a single transporter or if there are several transporters acting on one drug. Inhibition of the main efflux transporter would thus result in increased brain concentrations, while an interaction at an uptake transporter would decrease brain concentrations. In practice, it appears that interactions at the BBB are very rare, irrespective of the direction of active transport (Sadiq et al. 2011; Sasongko et al. 2005; Liu et al. 2008). This low incidence of interaction is possibly the result of relatively low concentrations of both victim drug and perpetrator in plasma. For example, the inhibition constant K_i for an interaction between diphenhydramine and oxycodone at the uptake transporter in cell cultures was much higher than the maximum possible clinical concentration (Sadiq et al. 2011). This is quite different from the situation in the gastrointestinal tract and the liver after oral administration, where much higher concentrations are present and the likelihood of an interaction is subsequently much greater.

7.6 Species Comparisons

Species differences in the extent of drug transport at the BBB are the result of differences in transporter expression and the capacity/specificity of substrates. It is well known that the expression of P-gp and BCRP proteins in humans is different from that in other species; for example, BCRP content is higher than P-gp content in humans, and P-gp content is higher than BCRP content in rats/mice (Ito et al. 2011; Uchida et al. 2011b, 2020). This could explain the differences in the results obtained when studying three PET tracers that are P-gp substrates in several species (Syvanen and Hammarlund-Udenaes 2010).

While the behavior of morphine at the BBB is very similar in rats, pigs, and humans ($K_{p,uu,brain}$ values are about 0.3–0.6), the $K_{p,uu,brain}$ in sheep deviates from this somewhat (1.2–1.9, depending on age) (Bengtsson et al. 2009; Ederoth et al. 2004; Tunblad et al. 2003, 2004a; Bouw et al. 2000;). This could be because of differences in transporter expression between sheep and the other species, i.e., possibly a lack of an efflux transporter.

There is a clear need for further translational studies between experimental animals and humans to learn more about species differences in transporter function at the BBB.

7.7 Current Status and Future Challenges

The understanding of the pharmacokinetics of drug delivery to the brain has developed rapidly, although there is still some confusion on rate vs extent measurements and methods and what they describe. There are today ways of measuring unbound concentrations in the brain using high-throughput methodology. In vivo studies have shown that there are still transport proteins acting as efflux or uptake transporters at the BBB that have not yet been identified. The presence and actions of transporters other than P-gp therefore need to be included in the thinking on brain penetration.

The scientific community and the drug industry are continuously striving to find correlations that will simplify measurements and enable prediction of successful new CNS drugs. There is, however, a difference between finding a correlation coefficient that is good enough versus predicting the fate of an individual compound based on this correlation or based on measuring a substitute parameter. The use of log-log comparisons and correlation coefficients could actually hide important information. Considering what we now know about individual BBB transport properties, it is actually easier to select new compounds that have high and low $K_{p,uu}$ values and assign them to potential clinical use depending on whether the desired effect is therapeutic efficacy or the avoidance of side effects in the CNS. Other aspects, such as peripheral side effects and affinity to target, are also included in the decision-making process. It is recommended to put as much effort into the decision on the kind of measurements to be made, as to put the efforts into finding correlations between measures that may or may not be clinically relevant. The area of BBB transport of drugs illustrates the time lag between new scientific findings and adoption of these findings in the drug industry. Shortening this time lag would significantly improve the success rate in drug discovery/development.

More research is needed before we can extrapolate information from animal studies to prediction of clinically relevant brain drug delivery. Some progress has recently been made in demonstrating the expression of transporters at the BBB for different species, but in vivo examples are needed to confirm these findings and more experimental studies are required. When we have identified most of the transporters, there is a real chance that predictive science will be able to help in the selection of good compounds for use in the CNS. There is also a need for better predictive disease models, understanding of disease mechanisms, and understanding of how disease states can influence drug transport into the brain, although these are beyond the scope of this chapter.

In an era of increased use of peptides and proteins, there is hope that some of these compounds will be available to the brain. The task before us, of understanding and improving their uptake into the brain from a quantitative and mechanistic perspective, is vast. A greater understanding and quantitative investigation of the role of nanocarrier delivery of drugs to the brain is also required. The achievement of successful delivery by these means in humans will require biocompatible carriers, and these should be a particular focus.

7.8 Conclusions

The rate and extent of drug delivery to the brain are two individual properties that are not numerically related. Data on intra-brain distribution are required to obtain the full brain delivery picture in relation to total (unbound plus bound) drug concentrations. The pharmacokinetic relationship between the permeability of the BBB (influx clearance) and the extent of drug delivery to the brain explains why the permeability per se is of lesser importance for brain drug delivery. Recent findings have confirmed the great value of focusing measurements on the extent of delivery of unbound drug to the brain. This is governed by the net flux of drug across the BBB and ultimately determines the clinical success rate when receptor occupancy is taken into account.

7.9 Points for Discussion

- What are the reasons for extent of delivery being more clinically relevant than rate of delivery for estimating the delivery of drugs into the brain?
- What are the essential processes governing the net influx and efflux clearances at the BBB, CL_{in}, and CL_{out}?
- For which purposes can $V_{u,brain}$ measurements be used?
- In what way could estimation of CNS exposure of drugs by the use of ratio of total brain to total plasma drug concentrations be flawed?
- How does the exchange of drugs between blood and CSF differ from the exchange between blood and brain ISF?
- How is the CSF concentration of the drug related to the brain interstitial fluid concentration? Discuss the rationale of using a surrogate approach for approximation of brain interstitial fluid concentration in preclinical and clinical studies (i.e., using other measurements than the direct ones).
- How may the understanding of intracellular distribution of drug contribute to establishment of a link between PK and PD?
- What are the clinically relevant sites of drug-drug interaction regarding brain drug delivery?
- What are the key components of interspecies differences in brain drug delivery?

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