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Review Article

Theme: Integrating Microdialysis and Imaging Tools in Systems Pharmacology
Guest Editors: Robert E. Stratford, Nimita Dave, and Richard F. Bergstrom

Microdialysis: the Key to Physiologically Based Model Prediction of Human CNS Target Site Concentrations

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Abstract. Despite the enormous research efforts that have been put into the development of central nervous system (CNS) drugs, the success rate in this area is still disappointing. To increase the successful rate in the clinical trials, first the problem of predicting human CNS drug distribution should be solved. As it is the unbound drug that equilibrates over membranes and is able to interact with targets, especially knowledge on unbound extracellular drug concentration-time profiles in different CNS compartments is important. The only technique able to provide such information *in vivo* is microdialysis. Also, obtaining CNS drug distribution data from human subjects is highly limited, and therefore, we have to rely on preclinical approaches combined with physiologically based pharmacokinetic (PBPK) modeling, taking unbound drug CNS concentrations into account. The next step is then to link local CNS pharmacokinetics to target interaction kinetics and CNS drug effects. In this review, system properties and small-molecule drug properties that together govern CNS drug distribution are summarized. Furthermore, the currently available approaches on prediction of CNS pharmacokinetics are discussed, including *in vitro*, *in vivo*, *ex vivo*, and *in silico* approaches, with special focus on the powerful combination of *in vivo* microdialysis and PBPK modeling. Also, sources of variability on drug kinetics in the CNS are discussed. Finally, remaining gaps and challenges are highlighted and future directions are suggested.

KEY WORDS: brain extracellular fluid ($brain_{ECF}$); central nervous system (CNS); cerebrospinal fluid (CSF); mastermind research approach; physiologically based pharmacokinetic (PBPK) model.

INTRODUCTION

There is a huge unmet medical need for central nervous system (CNS) disease therapies because of the growing of chronic and complex diseases associated with aging. However, development of CNS drugs is one of the most challenging tasks for the pharmaceutical industry (1). Actually, drug development for CNS drugs has suffered a higher attrition rate compared to that of other therapeutic areas drugs; it has been reported that only around 8–9% of CNS drugs that entered phase 1 were approved to launch (2). And around 50% of the attrition of potential CNS drugs has resulted due to a lack of efficacy and safety issues in phase 2 (2, 3). Knowledge of human CNS drug concentrations forms the basis for understanding exposure-response relationships; therefore, the lack of appropriate consideration of these target concentrations is one of the factors contributing to this high degree of attrition.

Obtaining the target site concentrations of CNS drugs is not straightforward because plasma concentrations do not adequately reflect CNS exposure, primarily due to the presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barriers (BCSFB), and additional specific physiological characteristics of the CNS. Furthermore, significant variation in the rate and extent of mechanisms that govern target site pharmacokinetics (PK), target engagement, and signal transduction is known to exist, due to differences in system conditions such as species, gender, genetic background, age, diet, disease, and drug treatment (4). Moreover, with regard to CNS drug action, there is a lack of sufficiently established clinical biomarkers and proof-of-concept (5). Thus, it is clear that there is a need for more predictive approaches. These predictive approaches have to be interconnected to the system conditions and must be performed using adequate (including bound and unbound drug)

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concentrations. Also processes should preferably not be studied in isolation and then combined, but instead studied in conjunction with each other as this will provide insight about the interdependencies of these processes (4). Since measurements on CNS target site concentration in the clinical setting are highly restricted, we have to develop an approach based on integrated preclinical data that is translatable to human.

Even though drug properties have been investigated well, information of CNS system properties (CNS physiology and biochemistry) is sparse and has a large variability. CNS pharmacokinetics of drugs is determined by their interaction. System properties depend on the condition of the system, which means that we have to use approaches to distinguish between system and drug properties, as this would allow us to translate the model to other species and also other disease conditions, by using physiologically based pharmacokinetic (PBPK) modeling.

Currently, many more or less complex semi-PBPK models have been published for CNS drug distribution. At present, four preclinical translational models have been validated with human CNS concentration profiles (6–9). In these models, however, the parameters were estimated using *in vivo* data to describe CNS distribution of individual drug in animals. Ultimate goal of the PBPK modeling is to build a generic PBPK model in which the parameters are derived from *in vitro* and/or *in silico* data. To achieve this, *in vivo* data is needed to validate the generic PBPK model. Furthermore, an investigation is needed on the relationship between drug physicochemical properties and CNS distribution.

In this review, system properties and small-molecule drug properties that together govern CNS drug distribution are summarized, followed by currently available approaches on prediction of CNS pharmacokinetics, including *in vitro*, *in vivo*, *ex vivo*, and *in silico* approaches, with special focus on the powerful combination of *in vivo* microdialysis and PBPK modeling. Also, sources of variability on drug kinetics in the CNS are discussed. Finally, remaining gaps and challenges will be discussed and future directions will be provided.

INTERACTION BETWEEN CNS SYSTEM AND DRUG PROPERTIES

Many CNS system properties and drug specific properties are known to influence drug kinetics in the brain, as shown in Fig. 1. Here, we focus on the relevant factors from each that contribute to the drug kinetics and summarize their function.

CNS SYSTEM PROPERTIES

Physiological Compartments, Flows, and pH

The CNS is a complex system composed of many physiological components and flows (Fig. 2): Physiological compartments are the BBB, the BCSFB, brain extracellular fluid ($brain_{ECF}$), cerebral blood, brain parenchymal cells, and the cerebrospinal fluid (CSF) in the ventricles, the cisterna magna, and the subarachnoid space (4). There are pH differences among the compartments (10–16). Then, there are the CNS fluid flows that include the cerebral blood flow (CBF), $brain_{ECF}$ bulk flow, and CSF flow. All relevant physiological parameter values are summarized in Table I.

Active Transporters

The localization of transporters and their expression level are also important factors to determine drug distribution in the brain. Transporters are present at the BBB and at the BCSFB, also on the membrane of brain parenchyma. Active transporters on the BBB and BCSFB consist of facilitated transport and ATP-dependent transport. The solute carrier (SLC) family, such as organic anion-transporting polypeptide (OATP) and organic anion

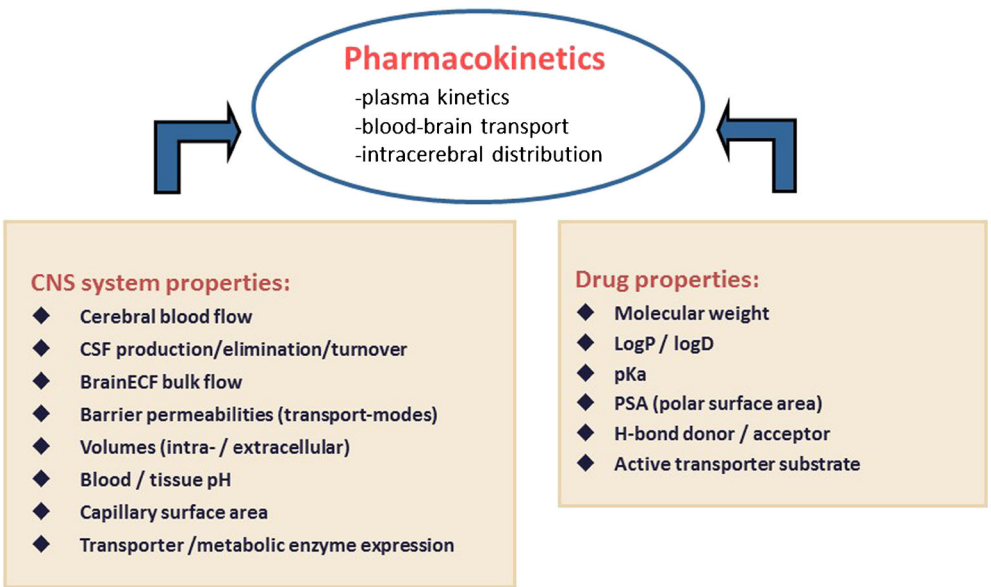

Fig. 1. System and drug properties which govern drug kinetics in brain. Figure is modified from de Lange (4)

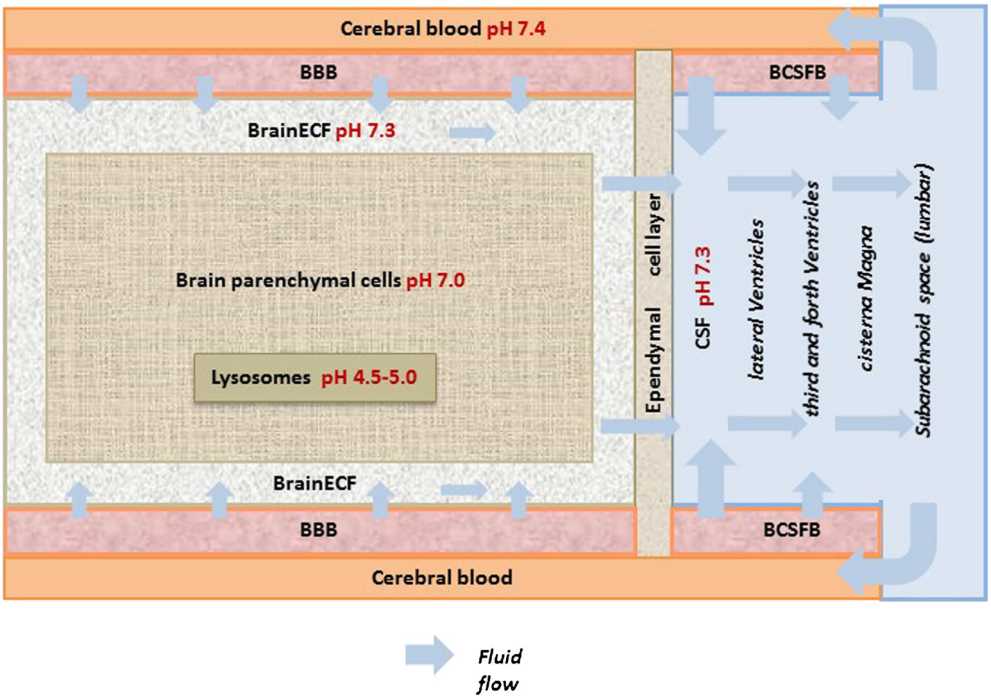

Fig. 2. Brain physiological components and flow. Figure is modified from de Lange (4)

transporters (OATs), is categorized as a facilitated transport, while ABC transporters, such as P-glycoprotein (P-gp), multidrug resistance protein (MRPs), and breast cancer-resistant protein (BCRP) are categorized as an ATP-

Table I. Values of CNS system properties for rat and human

	Parameter	Human	Refs	Rat	Refs
Volumes	BBB volume	8.25 mL (calculated using thickness endothelial cell of 550 nm)	(17)	5.02 μL	(18)
	BCSFB volume	107.25 mL (calculated using thickness 14.3 μm of endothelial cell)	(19)	37.5 μL	(18)
	Brain volume	1400 g	(20)	1.8 g, 1880 μL	(21,22)
	$Brain_{ECF}$ volume	240–280 mL	(23,24)	290 μL	(25)
	$Brain_{ICF}$ volume	960 mL	(24)	1440 μL	(24)
	CSF volume	130-150 mL	(26,27)	250 μL	(21)
	CSF_{LV} volume	20–25 mL	(26,28)	50 μL	(29,30)
	CSF_{TFV} volume	20–25 mL	(26,28)	50 μL	(29,30)
	CSF_{CM} volume	7.5 mL	(31,32)	17 μL	(31,32)
	CSF_{SAS} volume	90–125 mL	(26,28)	180 μL	(33,34)
Flows	cerebral blood flow	610–860 mL/min	(35–37)	1.1–1.3 mL/min	(38,39)
	$brain_{ECF}$ flow	0.15–0.2 mL/min, (50% of CSF production)	(27)	0.00018–0.00054 mL/min	(40)
	CSF flow	0.3–0.4 mL/min	(27)	0.0022 mL/min	(25,41)
Surfaces	BBB SA	12–18 m^2	(17)	155–263 cm^2	(42,43)
	BCSFB SA	6–9 m^2 (assumed 50% of BBB SA)	(17)	25–75 cm^2 (assumed 50% of BBB SA)	(42,44)
	brain ECF/ICF SA	228 m^2	Calculated [a)]	3000 cm^2	(18)
	brain ICF/lysosome SA	12 m^2	Calculated [a)]	162 cm^2	Calculated [a)]
pH	Plasma	7.4	(13)	7.4	(10)
	$Brain_{ECF}$	NA		7.3	(11)
	$Brain_{ICF}$	7.0	(14)	7.0	(11)
	lysosome	4.5-5.0	(15)	5.0	(11)
	CSF	7.3	(13)	7.3	(12)

$brain_{ECF}$, brain extracellular fluid compartment, $brain_{ICF}$ brain intracellular fluid compartment, CSF_{LV} compartment of cerebrospinal fluid in lateral ventricle, CSF_{TFV} compartment of cerebrospinal fluid in the third and fourth ventricle, CSF_{CM} a compartment of cerebrospinal fluid in the cisterna magna, CSF_{SAS} compartment of cerebrospinal fluid in the subarachnoid space, *SA* surface area

[a] Calculation was performed based on an assumption that the brain cells and lysosome are spherical

dependent transport (45). Table II summarizes an overview of transporters with their localization and their endogenous and exogenous substrates.

Metabolic Enzymes

Presence and localization of enzymes in the brain are also important factors to determine drug kinetics in the brain. In the brain, the following enzymes are found: oxidoreductases such as cytochrome P450 (CYPs) and monoamine oxidase (MAO), membrane-bound and soluble catechol-O-methyltransferase (COMT), and transferases such as uridine 5-diphospho (UDP) -glucuronosyltransferases (UGTs) and phenol sulfotransferase (PST) (68). In Table III, an overview is provided of the different enzymes with their localization and examples of their endogenous and exogenous substrates.

SMALL-MOLECULE DRUG PROPERTIES AND INTERACTION WITH THE CNS SYSTEM

A combination of CNS system properties and drug properties determines the pharmacokinetics of a drug in the CNS, including the CNS target site. Important physicochemical properties for determination of drug CNS pharmacokinetics are summarized in Fig. 1.

Physicochemical properties of a drug, such as lipophilicity, size, charge, hydrogen binding potential and polar surface area (PSA), are important determinants for pharmacokinetics of a drug. Many studies have investigated the influence of individual physicochemical properties on the BBB penetration in isolation. However, as physicochemical properties are highly inter-correlated, it is more appropriate to consider these properties in combination.

First of all, it should be noted that it is the unbound and neutral form of a drug molecules that is able to diffuse across barriers like the BBB and BCSFB, depending on the concentration gradient of the unbound and neutral form of the drug on either side of a membrane. Lipophilicity relates to the BBB permeability, as transcellular diffusion rate (93,94). Furthermore, as a rule of thumb, higher lipophilicity increases CNS tissue binding. Molecular size is an important factor for paracellular drug diffusion rate and also has an impact on transcellular diffusion rate at the BBB (93, 95, 96). The degree of ionization depends on the pKa of the drug and actual pH in a body compartment. Thus, the BBB permeability rate is influenced by lipophilicity, size, and pKa of a drug (93, 97). Using quantitative structure-activity relationship (QSAR) modeling, it has been shown that the descriptors for the prediction of BBB penetration are different for different charge classes (98). As there are pH differences between plasma, $brain_{ECF}$ and CSF (Fig. 2), charge is an important factor for CNS drug disposition (99).

The hydrogen bonding potential reflects the necessary energy for a molecule to move out of the aqueous phase into the lipid phase of a membrane. Recent studies have shown that the relationship between chemical structure and Kp,uu,brain (the ratio of the unbound concentration in the brain over that in plasma at equilibrium which measures the extent of CNS distribution) was dominated by hydrogen bonding (100).

PSA is generally defined as the sum of the van der Waals surface areas of oxygen and nitrogen atoms. Therefore, PSA of a compound can be related to its hydrogen bonding potential. Some studies have shown that PSA is highly correlated with the permeability coefficient (Pc) of membranes (94,101,102). A recent study for Kp,uu,brain has been shown that PSA is one of the important factors to predict the Kp,uu,brain for each compound (103).

BBB and BCSFB Transport

Protein Binding. It is generally accepted that unbound drug in plasma is able to cross the BBB and BCSFB. Two major proteins in plasma are albumin and α1-acid glycoprotein (104). For passive diffusion, the free concentration gradient between plasma and brain determines the rate of transport. The extent of BBB and BCSFB transport are investigated using Kp,uu,brain: If there is only diffusion, Kp,uu,brain is 1. If there is active transport processes, then Kp,uu,brain is larger than 1 (active in) or Kp,uu,brain is smaller than 1 (active out).

Ionization of the Drug in Plasma and in the Brain. There are similar pH differences among the CNS physiological compartments in human and in rat (Table I). Because of the pH differences, the ratio of neutral form of a compound among the compartments is different. It is generally accepted that neutral form can pass barriers; therefore, ionization that is determined by the pKa of a compounds and pH in the physiological compartments will have an impact on drug disposition in the brain.

Cerebral Blood Flow—Flow Versus Permeability-Limited Transport Rate. Lipophilic compounds usually have a large permeability coefficient; therefore, a permeability surface area product (PA), which is determined by the permeability coefficient and surface area of tissue, becomes large. If the PA is larger than the physiological cerebral blood flow, then the physiological cerebral blood flow determines the transport rate of the compound.

Modes of BBB Transport—Different Modes. The combination of transport modes at the BBB, BSCFB, and membrane of brain parenchyma determines the rate and extent of drug exchange at the BBB, BCSFB and membrane of brain parenchyma (105,106). Therefore, the operative transport mechanism(s) may differ for each drug. Each transport mode is summarized in Table IV.

Active Transporter Function. Active transporters mediate influx and efflux of drug transport. The magnitude of interaction of active transport is drug- and species-dependent (107). The functions of individual transporters are summarized in Table II.

Brain Distribution and Elimination

Extra-intracellular Distribution. Once having crossed the BBB, the drug is distributed by $brain_{ECF}$ bulk flow into the

Table II. Transporters in CNS

Transporter (gene name in human) (gene name in rat)	Tissue	Location			Substrates				Function	
		Human	Rat	Refs	Endogenous	Refs	Exogenous	Refs	Function	Refs
P-gp (ABCB1) (Abcb1a)	BBB	Luminal membrane of the BCEC	Luminal membrane of the BCEC	(46–48)	Cytokines	(49)	Antineoplastic agents, anticancer drugs, corticoids, analgesics, hydrophobic neutral or cationic compounds	(50)	Efflux	(51)
	BCSFB		Apical side of the CPEC	(52)					Influx/efflux	(52–54)
	BP	Adjacent pericytes and astrocytes	Astrocytes	(48,55)					Efflux	(55)
MRPs (ABCC1) (Abcc1)	BBB	Luminal and abluminal membranes of the BCEC	Luminal and abluminal membranes of the BCEC	(52,56–58)	Conjugated metabolites such as glutathione- and glucuronide-conjugates	(59)	Anticancer drugs, organic anion compounds, 17β-estradiol-d-17β-glucuronide	(49)	Efflux/influx	(54,60)
	BCSFB	Luminal and abluminal membranes of the CPEC	Luminal and abluminal membranes of the CPEC	(61,62)					Efflux	(63)
	BP	Astrocytes and microglial cells	Astrocytes and microglial cells	(60)						
OTAPs (SLCO, formerly SLC21A) (Slco1a/b)	BBB		Luminal and abluminal membranes of the BCEC (Oatp1a4 and Oatp1a5 and OATP2)	(45,64,65)	Amphipathic organ anions	(45)	Opioid peptides, E217bG	(66)	Efflux/influx	(61)
	BCSFB		Luminal membrane of the CPEC (Oatp1a4 and Oatp1a5, OATP2)	(45,64)						
			Brush border membrane of the CPEC (OATP1)	(45)						
OATs (SLC22A) (Slc22a)	BBB		Abluminal membrane of the BCEC	(45)	Organic anions	(67)		(67)	Efflux/influx	(45,61)
	BCSFB									

BBB blood-brain barrier, *BCSFB* blood-cerebrospinal fluid barrier, *BP* brain parenchymal cells, *BCEC* brain capillary endothelial cells, *CPEC* choroid plexus epithelial cells

Table III. Metabolic enzymes in the CNS

Human			Rat			Endogenous substrates	Refs	Exogenous substrates	Refs
Enzyme	Location	Refs	Enzyme	Location	Refs				
CYPs									
CYP1A1		(69)	CYP1A1		(69)	Melatonin, estradiol, arachidonic acid, progesterone, all-trans-retinal acid	(70)		
CYP1A2		(69)	(CYP1A2)		(69)				
CYP1B1	Cerebral microvessels at the BBB	(69,71)				Melatonin, estradiol	(70)		
			CYP2B		(69)	Arachidonic acid, testosterone, serotonin, anandamide, all-trans-retinoic acid,	(70)	Propofol	(72)
CYP2B6	Pyramidal neurons of the frontal cortex and astrocytes surrounding cerebral blood vessels	(69,73)				17-β estradiol, anandamide, arachidonic acid, estrone, serotonin, testosterone	(70)	Bupropion, diazepam, ketamine, methadone, meperidine, nicotine, pentobarbital, phencyclidine, propofol, sertraline selegiline, tramadol	(70)
CYP2C		(69)	CYP2C		(69)	Testosterone, progesterone, arachidonic acid, serotonin, harmaline, harmine, linoleic acid, melatonin, all-trans-retinoic acid	(70)		
			CYP2C13		(69)				
			CYP2D	Neuron, glia cells, choroid plexus	(74)	5-methoxytryptamine, octopamine, synephrine, tyramine, progesterone, anandamide, harmaline, harmine	(70)		
CYP2D6						5-methoxytryptamine, anandamide, progesterone, tyramine	(70)	Myltriptyline, brofaromine, clomipramine, codeine, citalopram, clozapine, desipramine, dextromethorphan, ethylmorphine, fluoxetine, fluvoxamine, haloperidol, hydrocodone, imipramine, mianserin, mirtazapine, nicergoline, nortryptaline, oxycodone, paroxetine, perphenazine, risperidone, tramadol, tranylcypromine, venlafaxine, zuclopenthixol	(75–77)
			CYP2D1		(69)				
			CYP2D18		(69)				
CYP2E		(69)							
			CYP2E1		(69)		(70)		(70)

Table III. (continued)

Human			Rat			Endogenous substrates	Refs	Exogenous substrates	Refs
Enzyme	Location	Refs	Enzyme	Location	Refs				
						Arachidonic acid, linoleic acid, oleic acid, 17-β estradiol, estrone, prostaglandin		Enflurane, felbamate, halothane, isoflurane, sevoflurane, trimethadione	
CYP3A		(69)	CYP3A		(69)				
CYP3A51		(69)							
			CYP4A		(69)				
			CYP4E		(69)				
COMT									
membrane-bound	prefrontal cortex	(78)	membrane-bound	prefrontal cortex	(78)	Dopamine	(79)		
soluble	prefrontal cortex	(78)	soluble	prefrontal cortex	(78)				
MAO									
MAOA	Adrenergic neurons	(80)	MAOA		(81–83)	Noradrenaline, adrenaline, dopamine, β-phenylethylamine and serotonin	(84)		
MAOB	Astrocytes and serotonergic neurons	(85)	MAOB		(81–83)				
UGT									
UGT2B7								Morphine	(86)
UGT1A6		(87)	UGT1A6		(88,89)				
Miscellaneous									
membrane-bound epoxide hydrolase		(87)							
benzoxyresorufin -0-deethylasetheir		(87)							
PST		(90–92)							

Table IV. Blood-brain barrier main modes of transport and their characteristics

BBB/BCSFB transport mode	Characteristics	Concentration-dependent transport kinetics?	Drug concentration-gradient dependent?	Consumes energy?
Paracellular	Passive; Between tight junctions of the BCEC and the CPEC	No	Yes	No
Transcellular	Passive; Across the membranes of the BCEC and the CPEC	No	Yes	No
Facilitated	Passive;	Yes	Yes	No
Active influx	Active;	Yes	No	Yes
Active efflux	Active;	Yes	No	Yes
Transcytosis	Receptor (specific, low capacity) or absorptive mediated (non-specific, high capacity)	No	No	Yes

BCEC brain capillary endothelial cells, *CPEC* choroid plexus epithelial cells

CSF compartments. At the same time, the drug in $brain_{ECF}$ is transported to brain parenchymal cell intracellular fluid ($brain_{ICF}$). It should be noted that also on the brain parenchyma cell membranes active transport may occur (106).

Tissue Binding. Tissue binding can occur as being specific at the target or non-specific to tissue components.

Lysosomal Trapping. In the brain parenchyma cells, there is a physiological pH gradient between the intracellular compartment (cytoplasm) and the lysosome compartment (Fig. 2). Especially basic compounds are known to be trapped in the lysosomes (11).

Drug Dispersion Within CSF. Some studies have shown that intrathecally administered drugs distribute faster than what can be accounted only by molecular diffusion (108, 109). Thus, it is thought that molecular diffusion makes only a small contribution to the total drug dispersion within CSF. This leads to the need to take into account also the convection due to oscillatory CSF flow to adequately explain this dispersion (110). Recently, the drug dispersion has been considered to be enhanced by the CSF pulsatility (heart rate and CSF stroke volume), and it leads to high inter- and intra-patient variability in drug distribution in the brain (110, 111).

Elimination from the Brain. Apart from transport across the BBB and BCSFB as discussed earlier, drug may leave the brain *via* the BBB, but also *via* CSF reflux into the blood stream at the level of the arachnoid villi.

Metabolism. In the brain, several metabolic enzymes are present. Enzyme interaction with drugs is important information not only on the drug PK profile but also the drug pharmacological effect in the brain since it may create active metabolites. Presence and localization of several enzymes have been reported in the brain (Table III), although their activity is reported to be relatively small compared to the liver (68, 87).

CURRENT APPROACHES TO INVESTIGATE CNS DRUG DISTRIBUTION

Since obtaining a human drug target site concentration in the brain is not feasible in most of the clinical studies, quantitative prediction of target site concentration is important. To achieve this, we need information from *in vitro*, *ex vivo*, *in vivo*, and *in silico* approaches. Here, we summarize the current approaches to obtain the necessary information to predict human drug target site concentration.

IN SILICO APPROACHES

For decades, QSAR studies have been performed using Kp,brain (total concentration ratio of the brain to plasma) or log BB, either of which may not reflect the relevant drug exposure in the brain to assess the efficacy of the drug since this efficacy is influenced by binding of compounds to plasma proteins and brain tissue. Eventually, log BB was replaced by the PA, as an estimate of the net BBB influx clearance (112). However, it has been argued that the PA cannot predict the unbound drug concentration in the CNS by itself. Recently, the most relevant parameter Kp,uu,brain has been used, with QSAR being conducted to model this parameter (100,103,113,114). Other than Kp,uu,brain, physiological meaningful parameter, Vu,brain (the volume of distribution of the unbound drug in the brain) or Kp,uu,cell (unbound concentration ration between $brain_{ECF}$ and $brain_{ICF}$) are also reported using molecular descriptors (103).

IN VITRO APPROACHES

In vitro approaches to investigate the BBB permeability have been conducted using BBB models (115). BBB models can be classified into non-cell based surrogate models, such as parallel artificial membrane permeability assay (PAMPA), and cell-based models such as primary cultures cells, immortalized brain endothelial cells, or human-derived stem cells (116). Although primary cultured cells from human tissue have been reported, acquiring human brain tissue is difficult as it can be

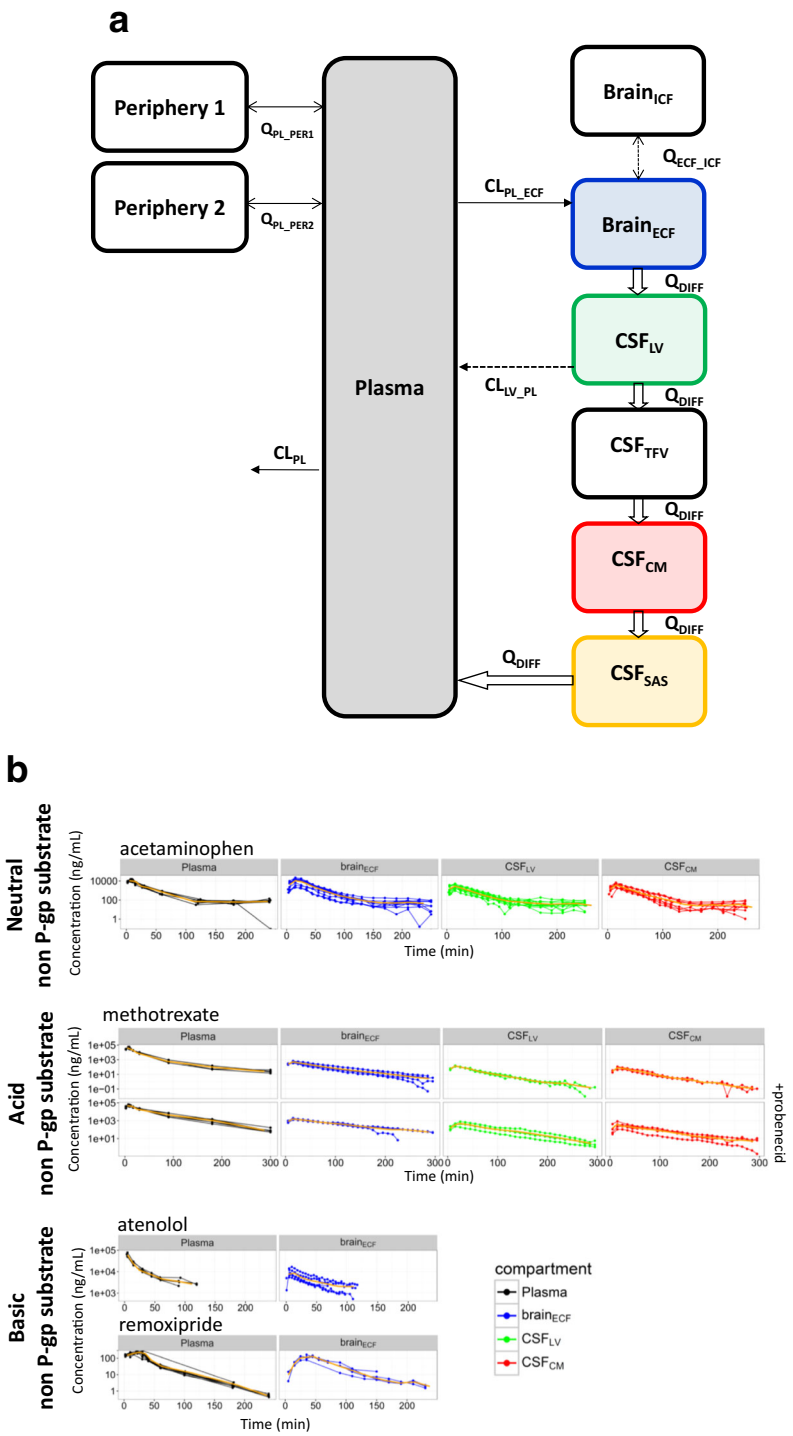

Fig. 3. A generic multi-compartmental CNS distribution model. (**a**) Multi-compartmental CNS distribution model structure. (**b**) and (**c**) Observations and model prediction (light orange solid lines) for the 9 compounds in rat for each dose and without and with co-administration of active transport blockers. The x-axis represents the time in minutes and the y-axis represents the concentration of the 9 compounds in ng/ml. The panel is stratified by brain compartments (*colors*) and by active transport blockers

obtained postmortem and should be fresh enough (117). Therefore, alternative models based on immortalized brain endothelial cells or human-derived stem cells are also used (118,119). Even though some models have been developed for measuring the BBB permeability, an ideal cell culture model of the BBB is yet to be developed. Furthermore, reliable *in vitro-in vivo* correlation data is needed to enable the use of *in vitro* results for the prediction of *in vivo* permeability. However, *in vitro* results have not been consistent in their ability to predict *in vivo* permeability, probably because of

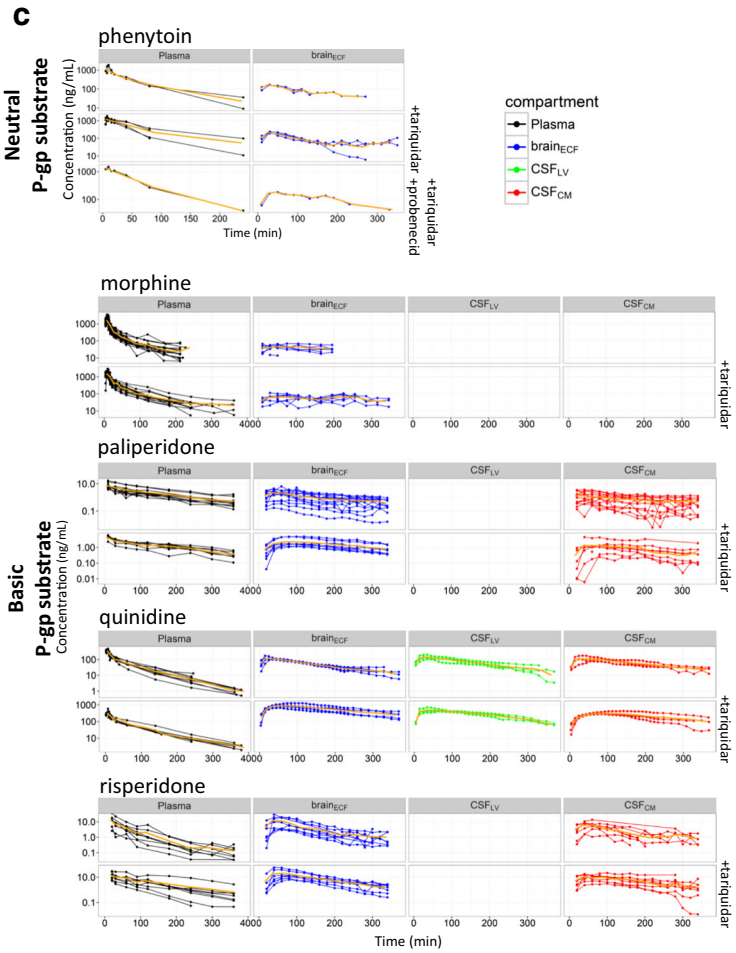

Fig. 3. (Continued)

different *in vitro* models and different sets of compounds used in the *in vitro* studies (120).

Currently, the biopharmaceutics classification system (BCS) and biopharmaceutics drug distribution classification system (BDDCS) are used for CNS drugs. The BDDCS is a modification of BCS that utilizes drug metabolism to predict drug disposition and potential drug-drug interactions in the brain (121). However, this classification approach needs to be further investigated because of inconsistencies. For example, it was proposed that 98% of BDDCS class 1 drugs would be able to get into the brain even though the drugs were P-gp substrates based on *in vitro* studies (122), while it has also been reported that the *in vitro* efflux ratio reflects the *in vivo* brain penetration regardless of the class in BDDCS (123).

EX VIVO APPROACHES

As mentioned before, it is the unbound drug molecules that are able to pass membranes and to interact with the target (21). Thus, measuring unbound drug concentrations is very important. Vu,brain or Fu,brain (the unbound fraction in the brain) is used to investigate unbound fraction of drugs in the brain. Fu,brain can be derived from brain homogenate (124), and Vu,brain can be obtained from the brain slice technique (125). The brain slice method is more physiologically relevant because the cell-cell interactions, pH gradients, and active transport systems are all conserved (114).

IN VIVO APPROACHES

Microdialysis can be considered as a key technique to time-dependent information regarding unbound drug concentrations. With microdialysis, both the rate and extent of drug transport and distribution processes can be determined (126,127). Thus, it can be used to obtain Kp,uu,brain in conjunction with the rate of transport processes. Moreover, this can be done at multiple locations, and this feature has shown that even for a drug like acetaminophen that is not subjected to any active transport, substantial differences in pharmacokinetic profiles exist in different brain compartments (6). While there is some limit to use this water-based technique for the highly lipophilic drugs, lots of microdialysis experiments have contributed to a boost in the understanding on drug exchange across the BBB (126,128,129). Especially the use of microdialysis at multiple brain locations has provided insight into the relative contribution of CNS distribution and elimination processes to the local (differences in) pharmacokinetics of a compound (6, 7, 130). It has paved the way to the development of a generic multi-compartmental CNS distribution

Table V. Sources of variability in CNS pharmacokinetics

Parameter	Location	Source of variability	Effect	Refs
Protein binding		Aging	Lower	(139)
		Pathophysiological condition	Higher with disease induced evaluation of plasma protein	(140,141)
Cerebral blood flow		Aging	Lower	(142)
		Pathophysiological condition	Lower in the multi-infarct group	(143)
		Diurnal variation	Change	(144)
BBB	Membrane lipid	Aging	Change	(145)
		Diet	Change	(146)
		Pathophysiological condition	Change in several disease conditions, such as Alzheimer's disease (AD) and schizophrenia	(147–149)
	Paracellular diffusion	Stress	Increase with hypoxic stress	(150)
		Pathophysiological condition	Increase (due to loose of tight junctions)	see below
	Tight junction	Pathophysiological condition	Disruption of the tight junctions by ischaemic brain stroke	(151)
			Opening of the tight junctions in AD patients	(152)
			Opening of tight junctions in multiple sclerosis patients	(153)
	Facilitated transport	Diet	Decreased in hypoglycemia condition	(154)
		Pathophysiological condition	Upregulation in the brain tumor	(155)
	Vesicle-based transport	Pathophysiological condition	Increase in experimental autoimmune encephalomyelitis	(156)
	Active transporters	Pathophysiological condition	See below	see below
$Brain_{ECF}$		Pathophysiological condition	Volume is enlarged in the patient with vasogenic type of brain	(157)
			Blockade of brain ECF flow in AD patient	(41)
Brain parenchyma		Aging	Shrunk	(158)
BCSFB		Aging	Thinner	(159)
		Pathophysiological condition	Decrease in Alzheimer patients	(159)
CSF		Aging	Decrease in CSF production, increase in CSF outflow resistance	(160)
		Pathophysiological condition	Decrease in CSF production, CSF Turnover and increase in CSF volume in AD patients	(161)
			Increased resistance to CSF absorption and CSF pressure in the patients with normal-pressure hydrocephalus	(162)
Brain metabolic enzymes		Aging	Increase in the CYP2D6 enzyme level	(163)
		Gender	Higher MAO activity in women	(83)
		Pathophysiological condition	Higher MAOB activity in AD patients	(164)
			Difference of COMT expression in schizophrenia patients	(78)
		Gene	Deficiency of CYP2D6 enzyme	(75)
			Change of COMT function	(165,166)
		Smoking and alcoholism	Change of CYP2B6 and CYP2E1 levels	(73,167)
Transporter		Aging	Decrease in P-gp activity	(168)
			Decrease in glucose transporter activity	(169)
		Pathophysiological condition	Upregulation of P-gp and MRPs in epileptogenic brain	(170)
			Upregulation of P-gp and MRP1 in the brain tumor	(171)
			Alteration of the levels of glutamate transporter in the various brain disorders, including cerebral ischemia, amyotrophic lateral sclerosis, AD, AIDs, traumatic brain injury, schizophrenia, and epilepsy (seizure)	(172,173)
		Diurnal variation	Change in P-gp activity	(174)

Table VI. Currently published (semi-) PBPK model for CNS drugs

Model structure								Data								Model parameters	Translation		Refs
Plasma	$brain_{ICF}$	$brain_{ISF}$	total brain or brain cell	CSF (general)	CSF_{LV}	CSF_{CM}	CSF_{SAS}	Drug(s)	Species	Plasma	$brain_{ICF}$	brain homogenate	CSF general	CSF_{LV}	CSF_{CM}	CSF_{SAS}		Simulation only	Validation with human data	
								not specified	human								system and drug properties			175
								quinolone	rat								estimation			176
								3'-Azido-3'-Deoxythymidine, 2',3'-Dideoxyinosine	rat								estimation			177
								caffeine, mannitol, tryptophan, 3 anonymous compounds	rat								estimation			178
								morphine-6-h-d-glucuronide	rat								estimation			179
								caffeine, CP-141938, fluoxetine, NFPS, propranolol, theobromine, theophylline	rat								system properties, *ex vivo* data (*in situ* brain perfusion and equilibrium dialysis)			180
								atomoxetine, duloxetine	rat											181
								domperidone	mice								system properties and *in vitro* data			182
								Morphine, oxycodone	rat								system properties and *in vitro* data			183
								acetaminophen	rat								estimation			6
								quinidine	rat								estimation			59
								methotrexate	rat								estimation			7
								not specified	mice, rat								system properties and *in vitro* data			184
								2 anonymous compounds	rat								system properties and *in vitro* data			77
								acetaminophen, phenytoin	human								system properties and *in vitro* data, estimation of active transport impact			8
								acetaminophen, atenolol, , methotrexate, morphine, paliperidone, phenytoin, quinidine, remoxipride, and risperidone	rat								estimation			9

The green boxes represent which physiological compartments were taken into account in each model structure. The blue boxes show which compartment data was used for each modeling if *in vivo* data was used. The red boxes explain what translational research was performed with each model if applicable

model (Fig. 3), with some validated human CNS predictions that will be discussed later in this review.

Then, positron emission tomography (PET) is a valuable non-invasive *in vivo* monitoring technique that can be used to visualize drug CNS distribution in living animals and human. However, the PET technique cannot distinguish parent compounds from their metabolites or bound and unbound drug. Furthermore, it may also encounter difficulties in obtaining useful data when a very high non-specific binding (NSB) to non-target proteins and phospholipid membranes occurs (131). Recently, a novel lipid membrane binding assay (LIMBA) was established as a fast and reliable tool for identifying compounds with unfavorably high NSB in the brain tissue (132).

COMBINATORY MAPPING APPROACH

Combinatory mapping is an approach that combines three compound-specific parameters obtained from *in vitro*, *ex vivo*, and *in vivo* data: Kp,brain, Vu,brain, and Fu,plasma, for calculation of Kp,uu,brain (133). This approach also can be used to obtain not only Kp,uu,brain but also to understand unbound drug disposition in the cell cytosol and the lysosomes. Recently, this approach has been extended to predict drug exposure in different brain regions such as frontal cortex, striatum, hippocampus, brainstem, cerebellum, and hypothalamus, in which also the impact of transporters and receptors in each region was taken into account (134). Although this approach is useful to support the selection of potential CNS drugs in drug discovery, it has two limitations. The first limitation is that it can only predict the parameters at steady-state. The second limitation is that the approach cannot be translated to predict the parameters, for instance, inter-species or inter-disease conditions because the processes to obtain the parameters in this approach are not connected with system properties which will be changed in these conditions.

CONDITION DEPENDENCY AND PBPK MODELING

Condition Dependency

Drug distribution into and within the brain depends on the interaction between system and drug properties. Drug properties remain the same, whatever the species and conditions are in which the drug has been administered. This indicates that interspecies variability in drug distribution into and within the brain is the result of differences in physiological and biochemical parameters. Factors which cause variation in drug pharmacokinetics include genetic background, species differences, gender, age, diet, disease states, and drug treatment (4). Factors which cause variation in drug pharmacodynamics include seasonal

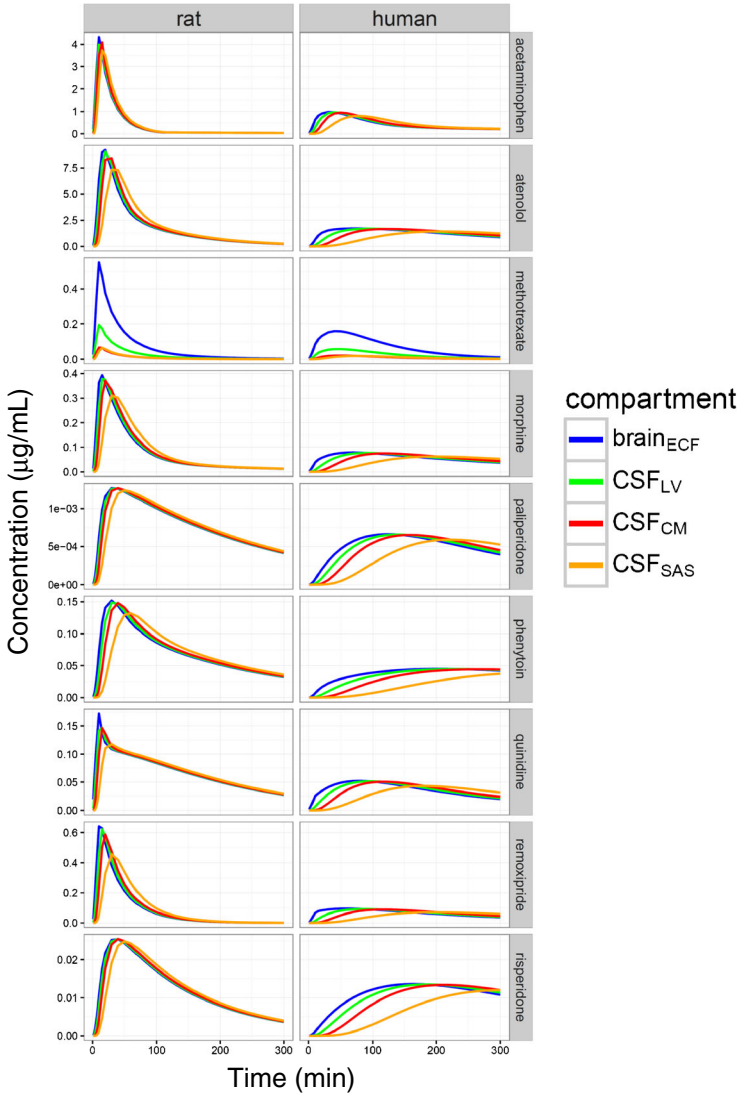

Fig. 4. Simulation of time concentration profiles of brain$_{ECF}$, CSF$_{LV}$, CSF$_{CM}$ and CSF$_{SAS}$ in rat and human for the 9 compounds. Differences in the time concentration between rat and human reflected the differences of the rate and extent of drug distribution in brain between in rat and in human since identical plasma exposure was used as an input

effect (135), age (136), gender (137), and species (138). Influences of these conditions on CNS system properties are summarized in Table V.

(Semi-) PBPK Modeling

PBPK models need to be informed on system and on drug properties to model the interaction and predict the PK in different compartments. Especially as obtaining pharmacokinetic data from the human brain is highly restricted, working in the PBPK model framework is valuable as it can be translated to predict the target site concentrations in inter-species and inter-disease situations (4). Some translational studied have been reported by using an animal (semi-) PBPK model for CNS drugs but they are relatively sparse and range from simple to more advanced (Table VI).

Recently, a generic multi-compartmental CNS distribution model structure has been proposed, that could successfully describe the pharmacokinetics in plasma and different CNS compartments (brain$_{ECF}$, CSF in the lateral ventricle (CSF$_{LV}$) and CSF in the cisterna magna (CSF$_{CM}$)), using microdialysis data for 9 paradigm compounds with substantial differences in physicochemical properties (9) (Table VI, Fig. 3). These compounds are acetaminophen, atenolol, methotrexate, morphine, paliperidone, phenytoin, quinidine, remoxipride, and risperidone. This is the first model that can nicely predict human brain$_{ECF}$ and CSF time concentration profiles which were obtained from physiologically "close to normal" brain for morphine and acetaminophen (9).

For remoxipride, Stevens *et al.* have shown that brain$_{ECF}$ pharmacokinetics, as measured with microdialysis, represented the target site concentrations, because these concentrations could be directly linked to the effect of remoxipride

on plasma prolactin levels in an advanced mechanism-based model (185). After scaling to human, this indeed could also be concluded for human CNS remoxipride effects on human plasma prolactin levels. This underscores the importance of having information on pharmacokinetics at the CNS target region.

Using our generic multi-compartmental CNS distribution model, we can provide predictions of human CNS pharmacokinetics for all the nine compounds. For a direct comparison of rat and human pharmacokinetics in the different CNS compartments in response to plasma pharmacokinetics, the same plasma exposure was used for individual compound. In Fig. 4, it can be seen that, in general, human CNS pharmacokinetics, especially that in the CSF in the subarachnoid space (CSF_{SAS}), which is including the lumbar CSF is typically slower than that in the rat. This provides important information on the relationship between $brain_{ECF}$ (which often is the target site) pharmacokinetics and the lumbar CSF concentrations that are often used as biomarker of brain target site concentrations. Also, it can be seen that the differences in the pharmacokinetics at the more early time points of the different CNS compartments is larger in human than in the rat. With time, these differences fade out. The consequences for drug-target interaction kinetics (186) and further processes towards CNS drug effects remain to be determined.

Remaining Gaps and Challenges on PBPK Modeling, Towards a Generic PBPK Model

The ultimate aim is to have a CNS PBPK model that can predict human brain compartment concentrations on the basis of the physicochemical properties of a compound, which can be determined by *in vitro* measurements, or *in silico* prediction. Thus, in the overview in Table VI, it can be seen that we still have a number of gaps in the currently available (semi-) PBPK models of CNS drugs. Most of the models require *in vivo* data on the compound(s), and most of the predictions have not been validated on human data. Even the most comprehensive model (9), with validated prediction of human CNS drug distribution (for acetaminophen and morphine), still requires *in vivo* data for individual compound predictions. Thus, it can be seen that there is a need for further development of a generic, fully PBPK model for CNS drug distribution (187–189).

To have a PBPK model that would predict CNS drug distribution, based the physicochemical properties of an individual drug, for different species and in different conditions, a number of challenges remain:

- Having a PBPK model structure with all relevant compartment/parameters, as physiological parameter values reported are sparse and variable (see Table I).
- Having drug physicochemical parameter values from *in vitro*, and/or *in silico*, or even some *in vivo* measurements, which may not necessarily be correct. For example, *in vitro* or *in vivo* data may depend on the experimental setting, while *in silico* information really depends on the data availability, used to obtain the equation.
- Having human data sets for validation of prediction by the model, with typically limited availability.
- Having information on pathophysiological changes in human CNS properties in (the many) disease conditions. For example, BBB characteristics may change in Alzheimer's disease, multiple sclerosis, and pharmacoresistant epilepsies (190).

DISCUSSION AND CONCLUSION

Pharmacokinetics of drugs in the CNS is governed by a combination of CNS system physiology and drug properties. This means that variability in CNS system physiological parameters (condition dependency) may lead to variability of CNS pharmacokinetics. Therefore, it is important to explicitly distinguish between system physiology and drug properties, either by changing conditions and investigating the pharmacokinetics of one drug, or investigating the pharmacokinetics of different drugs in the same condition.

PBPK models make this distinction; however, being based on total drug plasma and total tissue concentrations at equilibrium (SS), while more recent PBPK models include, at best, unbound plasma SS concentrations. However, as body processes are based on the interaction with the unbound drug and are time-dependent, it is crucial to include measuring the unbound drug in each compartment as a function of time (Mastermind Research Approach (MRA)) (4), for which microdialysis has been proven the key technique. Using the MRA, microdialysis has provided lots of valuable data that pave the way towards a semi-physiological generic CNS drug distribution model, yet applicable for nine compounds with highly different physicochemical properties with excellent description of the rat data for all these compounds, and adequate prediction of human CNS data that were available for acetaminophen and morphine (9).

One microdialysis experiment in a single freely moving animal can provide a lot of data points, obtained under the same experimental condition of the animal, and thereby revealing the interrelationships of processes. With this microdialysis has already contributed to reduction and refinement in the use of animals. Furthermore, all this information can further be "condensed" into a generic PBPK model and will thereby help in the reduction in the future use of animals (replacement) (191).

So, in order to be able to predict CNS drug effects in human, next steps would be a development of a full PBPK CNS drug distribution model, and combine it with target binding kinetics, receptor occupancy, and signal transduction (186,192), and include system changes by human disease condition.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

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