TOPICAL REVIEW

Physical insights into the blood–brain barrier translocation mechanisms

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Abstract
The number of individuals suffering from diseases of the central nervous system (CNS) is growing with an aging population. While candidate drugs for many of these diseases are available, most of these pharmaceutical agents cannot reach the brain rendering most of the drug therapies that target the CNS inefficient. The reason is the blood–brain barrier (BBB), a complex and dynamic interface that controls the influx and efflux of substances through a number of different translocation mechanisms. Here, we present these mechanisms providing, also, the necessary background related to the morphology and various characteristics of the BBB. Moreover, we discuss various numerical and simulation approaches used to study the BBB, and possible future directions based on multi-scale methods. We anticipate that this review will motivate multi-disciplinary research on the BBB aiming at the design of effective drug therapies.

1. Introduction

Millions of people around the world suffer from some kind of central nervous system (CNS) disorder at a certain time of their life, which is believed to be the case for one out of three individuals [1, 2]. It is projected that approximately two billion people will experience some type of CNS disorder by 2020, if we do not develop new treatments [3]. As an example, the annual expenditure for Alzheimer’s Disease (AD) in the USA alone could reach as much as $0.5 trillion by 2020; in part, due to the increase by 50% of the population of people over 65 years old [2]. In order to cure diseases of the CNS, however, we need to deliver drugs to the brain. In fact, more than 98% of small drug molecules (e.g. lipid-soluble small molecules with a molecular weight less than approximately 400 Da [2]) and almost all of large drug molecules are unable to be delivered to the brain through the body circulatory systems [4, 5], mainly due to the presence of the blood–brain barrier (BBB). To this end, the BBB has various roles. For example, it regulates the micro-environment of the brain by separating it from the periphery. Moreover, it guarantees its proper function in healthy humans by protecting the brain from toxins and pathogens, but allowing for the delivery of necessary nutrients and oxygen to the brain. Also, it participates in the maintenance of the CNS homeostasis while serving as an interface for communication between the periphery and the brain [6–9]. To this end, the BBB dysfunction is relevant to many pathologies [6, 10], e.g. stroke [11–14], trauma [15, 16], infectious or inflammatory processes (e.g. meningitis) [17, 18], multiple sclerosis [19, 20], HIV [21, 22], Alzheimer’s disease [23–27], Parkinson’s disease [25, 28], epilepsy [29, 30], brain tumors [31–34], pain [35], glaucoma [36], anorexia [37], obesity [38], migraine [39], lysosomal storage diseases [40], and others.

The BBB is a complex, dynamic and adaptable interface formed by thin capillary endothelial cells (ECs) surrounded by a number of different cells that provide functional and structural support (figure 1), such as pericytes and astrocytes, which together with neurons, microglial cells and, occasionally, peripheral immune cells form the so-called neurovascular unit (NVU) [8, 41–44]. For ECs of the BBB, the distance between lumenal and ablumenal membranes is approximately 250 nm [6–8, 45–49], 39% thinner than the muscle ECs [45, 50]. The BBB exists at all levels of the vascular tree within the CNS [7, 46, 51, 52] and acts as a selective barrier for the exchange of substances between the blood microcirculation system and the

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brain parenchyma through a number of translocation mechanisms [49, 53]. There are about 100 billion such capillaries with an average distance between them of 40 μm, a combined total length of 650 km, and a drug transport surface area of approximately 12–18 m² per kilogram of brain for an average human adult [54–56]. The brain itself does not have the ability of storing substances such as oxygen or glucose. Therefore, it receives through these capillaries approximately up to 20% of the cardiac output and 25% of oxygen consumption despite its 2% weight. In this way, the brain satisfies its high metabolic activity [47, 57–59]. A proper blood flow through the capillaries is very important, otherwise brain functions could stop in seconds and damages to neurons may occur in minutes [47, 60]. Although other pathways for delivering substances to the brain exist (e.g. the blood–cerebrospinal fluid barrier) [61], the BBB is the one that has the largest surface area for providing nutrients (e.g. 5000 times larger surface area than the blood–cerebrospinal fluid barrier) [6, 8, 59]. Moreover, the distance between individual neurons and the nearest brain capillary is about 10 μm, whereas the distance between the cerebrospinal fluid compartment and neurons is of the order of millimetres or even centimetres [41, 62]. The BBB has a vital importance in regulating the function of the CNS, while the development of therapeutics for CNS disorders depends on the ability of delivering chemical agents through the BBB to the brain [49]. Despite the importance of understanding this complex interface, there is still little research apart from the traditional in vivo methods [2]. Overcoming the BBB requires the understanding of the translocation mechanisms that control the passage of substances through the BBB [49, 63]. In this regard, in silico models offer particular advantages over experimental methods. For example, they are inexpensive, faster, and do not require animal testing or the use of large compounds [4, 64]. Most of the computational methods developed for predicting the permeability of the BBB are based on the structure activity relation (SAR), statistical analysis, artificial intelligence and machine learning, genetic algorithms, and neural networks [4]. Notwithstanding, there is the potential for a significant contribution from molecular and continuum-scale models that could provide the basis for addressing important physical questions regarding the BBB, for example, understanding the dynamic equilibrium of this complex interface.

The aim of this review is to introduce the BBB to a broader biophysics community, present the main translocation mechanisms and recent computational and numerical studies associated with it, and discuss opportunities for new directions and modeling approaches to be employed for the study of the BBB using novel multi-scale modeling and simulation methods.

2. Historical perspective of the blood–brain barrier

In 1885, Ehrlich injected water-soluble dyes in the peripheral circulation [65]. He found that the dyes stained several tissues (e.g. choroid plexus) except for the brain and the cerebrospinal fluid (CSF) attributing his finding to the low affinity of these dyes to the CNS [65–67]. On the contrary, injection of the same dyes in the subarachnoid space stained the brain and the CSF, but not the peripheral tissues [68]. These experiments contributed to the concept of the barrier between blood and cerebrospinal fluid compartment at the choroid plexus, and the blood and the brain at the cerebral microvasculature [69]. Further experiments confirmed the existence of a barrier between the brain parenchyma and blood [70], and identified the physiological function of this barrier in maintaining the brain homeostasis [64, 67, 71]. However, it was the advancement of electron microscopy and studies performed in the late 1960s [72, 73] that lead to the conclusion that the BBB consists of non-fenestrated ECs linked with tight junctions (TJs). It has been known for the last three decades that the BBB ECs have unique physiological characteristics compared with other ECs in peripheral tissues [74]. A discussion of the different critical experiments on the way to coining the ‘BBB’ term can be found in [57]. A number of recent studies have provided valuable information regarding the structure and function of the BBB and its role in various diseases [49]. Based on this knowledge in silico and theoretical modelling have emerged as important tools towards understanding the physical properties of the BBB to be employed in drug design.

3. Morphology and structural components of the blood–brain barrier

The main anatomical and functional part of the BBB is the cerebral ECs that completely encircle the lumen (figure 1) [59]. Extracellular base membrane [45, 47, 64, 75–87], perivascular/periendothelial cells (pericytes [25, 45, 47, 49, 50, 64, 76, 78, 85, 88–98, 99–114] and astrocytes [32, 41, 45, 47, 49, 72, 78, 115–125, 126–144]), and microglia [45, 47, 69, 78, 104, 145–152] are located on the abluminal surface of the ECs being an integral part of the BBB supporting system. The basal lamina [45, 47, 64, 75–87] surrounds the ECs and pericytes, with the region between them known as the Virchow–Robin space. Astrocytes are adjacent to the ECs, with astrocytic end-feet sharing the basal lamina. Together with neurons and microglia, they form a complex and functional NVU [7, 45, 47, 59, 69, 76, 78, 104, 145–153]. The properties of the BBB are mainly determined by the ECs, but are induced and maintained by complex communication processes with the other type of cells according to the
needs of the CNS [45, 49, 76, 141, 142, 154]. The BBB has certain characteristics that gives it unique properties [59]. Examples of these features are the lack of fenestrae, the small number of pinocytic vesicles, the large number and volume of mitochondria in ECs [155–157], the presence of TJ formed by transmembrane and adhesion proteins (junctional adhesion molecules (JAMs)) and Adherens junctions (AJs) [6, 35, 41, 45, 47, 57, 59, 64, 67, 71, 72, 76, 78, 85, 93, 101, 119, 158–168, 169–187] that can be regulated through communication with the periendothelial cells [85, 114, 188, 189], the expression of various transporters that prevent or facilitate the entrance of substances to the brain [41, 190], and a strict limit for the passage of immune cells (especially lymphocytes) [150, 191–193]. All these characteristics grant BBB multiple functions as a physical, transport, metabolic or enzymatic (specialised enzyme systems) [194] and immunological barrier [59]. The BBB also acts as a dynamic interface that regulates the CNS homeostasis [45].

ECs are mesodermally derived simple squamous epithelial cells forming the walls of the blood vessels with intercellular clefts being of the order of 200 nm [45]. Although blood vessels with large diameters can be made up of a dozen of ECs, the smallest capillaries can be formed by a single endothelial cell folded to itself [45, 51, 52]. The apical (lumenal) and basolateral membranes (BM) are functionally different due to their distinct composition, including different levels of protein and level compositions, enzymes, and transporters [49, 78, 195–197]. Moreover, ECs of the BBB lack fenestrations and TJ seal cell-to-cell contacts between adjacent ECs forming a continuous blood vessel, which results in high electrical resistance (in the range 1500–2000 Ω cm² compared to a few Ω cm² in other tissues [198]) in this way preserving cell polarity and cell adhesion [6, 45, 64, 67, 72, 73, 86, 119, 160, 164, 185, 198–202], and restricting the amount of paracellular flux for larger solutes [45, 72, 73, 78, 101, 158, 159]. Under normal conditions, uncharged molecules up to an approximate molecular weight of 180 Da can selectively pass through the TJ [76, 203–205], while for larger molecules there exist a specialized transport system needed to transfer various substances to the brain [101, 158, 159]. Moreover, cerebral ECs possess higher number and volume of mitochondria suggesting high metabolic activity [45, 47, 78, 101, 157–159, 194], which is necessary for transporting molecules against concentration gradients [71].

4. Molecular properties and integrity of the BBB

The BBB regulates homeostasis of the neural microenvironment, transports nutrients and other molecules required by the CNS (e.g. vitamins) and provides protection from toxins in order to contribute to proper neural function [6, 41, 45, 67]. At the same time, BBB is also regulated by interaction with the microenvironment of the CNS [45, 74, 206], e.g. through a number of proteins, inflammatory mediators, free radicals, microRNAs and others [53].
The local ionic microenvironment of the synapses and axons is protected from fluctuations in the blood composition due to food intake or physical exercise [207]. Despite these changes, the BBB provides ion regulation and high resistance paracellular barrier to small hydrophilic molecules (e.g. due to the presence of TJs) [208–212], while it ensures the passage of important water-soluble nutrients and metabolites required for the brain nutrition by employing specific transport mechanisms [207]. For example, water-soluble molecules can be transported up to two orders of magnitude faster than the predicted rate based on physicochemical characteristics (e.g. molecular weight, lipophilicity, and others) [213]. Moreover, the BBB controls the molecular traffic by regulating the entrance of macromolecules to the brain, mediating efflux of many waste products, minimising neuronal cell death, and preserving neural connectivity [6, 207]. Also, the BBB impedes the exchange of proteins between the luminal and abluminal membranes in this way maintaining a low protein environment in the CNS. In addition, the BBB allows for non-synaptic signaling in the CNS while separating the CNS and the peripheral neurotransmitter pools [207].

A dynamic equilibrium of all these processes is controlled by the BBB, which acts as a complex, dynamic and adaptable interface (physical barrier) between the blood and the brain. In order to have such a role, the BBB possesses certain mechanisms for translocating various molecules from the blood towards the brain, as well as from the brain towards the blood [207]. In the following, we discuss these mechanisms, which are important components to be considered in building physical models that can mimic the behaviour of the BBB with the focus being on the ECs.

5. Translocation mechanisms: fundamental description and recent advances

The transport of substances across the BBB can occur through the paracellular (between adjacent cells) or the transcellular pathway (through the cells), both of which are non-saturable and non-competitive (see figure 2, which illustrates schematically various translocation mechanisms of the BBB) [6,8,49,64,214–217]. This may be an important feature for modeling independently these processes. In the case of paracellular passage, ions and solutes diffuse between the cells according to their concentration and electrostatic gradient (passive diffusion) [166]. In the case of the transcellular pathway there are different mechanisms, such as passive or lipid-mediated diffusion and cell migration (diapedesis), as well as mechanisms based on specific transporters (active mechanisms), such as carrier-mediated transport (CMT), receptor-mediated transcytosis (RMT), adsorptive-mediated transcytosis (AMT), and active efflux transport (AET) [41, 64, 218]. All these mechanisms, which can be also broadly categorised as passive and active processes (i.e. energy is required in order to transfer the substance through the BBB), regulate the transport of substances between the blood and the brain.

Here, we provide a few examples of molecules associated with different translocation mechanisms. For example, small water-soluble agents may use the paracellular route [219]. Other small molecules, such as oxygen, ethanol, and CO₂, can cross the BBB by diffusion through the ECs membranes, but can be exposed to eflux pumps (e.g. P-glycoprotein) [63, 64]. Moreover, small hydrophilic molecules and small hydrophilic nutrients may use specific transporters expressed at the luminal (blood) and basolateral (brain) side of the ECs [63]. Active transporters, such as glucose transporter-1 (GLUT-1) and ATP-binding cassette(ABC) transporters being present on both luminal and abluminal membranes and can act as influx and efflux transporters at the expense of energy coming from ATP [41, 102, 122, 220], may be also used for the transport of proteins and peptides. Nutrients cross the BBB through passive diffusion of lipid soluble compounds, CMT and RMT [8]. Harmful metabolites from xenobiotics and drugs are pumped out by active efflux transporters (e.g. AET) [8]. Generally, the entry to the CNS depends on a number of factors, such as molecular weight (MW), concentration gradient, amino acid composition, lipid solubility, hydrogen bonding, charge, aggregation, and 3D structure (e.g. conformation, flexibility, folding, affinity for eflux proteins, receptors and carriers, cellular enzymatic activity) [1]. Additional factors, such as lipid composition of the ECs, membrane charge, and peripheral factors, such as affinity for plasma proteins, systemic enzymatic stability, cerebral blood flow, volume of distribution of substances, may also play a role [1]. Therefore, the characteristics of the chemical agents and the physical properties of the BBB determine whether a substance will enter the brain [221].

Most of the discussion in this review applies for normal conditions and does not consider the situation of microbial infection or conditions that affect the equilibrium properties of the BBB. It is expected that the behaviour of the BBB may change drastically in situations, such as microbial infection, where the integrity of the TJs or the supporting components of the TJs is affected [180, 222–225].

5.1. Paracellular diffusion

Paracellular diffusion is a passive process for water and small hydrophilic solutes for the transport through the ECs intercellular space and TJs [57, 101, 226]. It may depend on the electrochemical, hydrostatic and osmotic gradients, and solute concentration being structurally mediated by the TJs [101]. To this end endothelial cytoskeleton contractility and adhesive forces affect the function of TJs and eventually the opening and closing of the paracellular pathway [101, 227, 228]. In general, large changes are observed at the time of lowest transendothelial electrical resistance.
and highest permeability coefficients for tracers (e.g. mannitol) [180]. However, the exact mechanism of these processes in association with the intraendothelial contractile forces requires further investigation [180]. The paracellular route is generally exploited by small molecules and exploitation of this mechanism for drug delivery requires the opening of the intercellular cleft for the passage of larger substances [101, 229].

5.2. Passive diffusion
Small lipid soluble compounds may passively diffuse through the BBB from high to low concentrations by dissolving in the lipid plasma membrane (transendothelial diffusion) [49]. In general, the more lipophilic a substance the higher is the probability of penetrating the BBB [59, 230], as it correlates with the rate of entering the substance into the brain, which can be determined in silico [6, 231, 232]. Due to this property, liposomes have been suggested as an approach for drug delivery [1]. Apart from physicochemical characteristics of the substances, other factors, such as the flow dependence as the concentration of the substance differs along the blood capillary may also play a role [49].

In general for membranes, passive diffusion can be considered as a spontaneous process where the free-energy change of a diffusing solute across a membrane depends on the concentration gradient [233]. In the absence of charge (non-electrolyte solute), the free-energy difference is given by

$$\Delta G = RT \ln \frac{C_i}{C_o}, \tag{1}$$

where $R$ is the gas constant, $T$ the absolute temperature, and $C_i / C_o$ is the ratio of the solute concentration on the inside (i) and outside (o) membrane surfaces of the ECs [233]. This ratio determines whether the net influx of the solute is thermodynamically favoured (exergenic), which takes place when $\Delta G$ is negative, i.e. ratio smaller than one. If the external concentration of solute is tenfold higher than the internal at room temperature, then $\Delta G = -1.4$ kcal mole$^{-1}$, which is the energetic cost of maintaining a tenfold concentration gradient [233].

In the case of charged species, one should also take into account the overall charge difference between the two compartments. Like charges of the solute and the compartment do not favour the translocation of the molecules, whereas difference in net charge favours thermodynamically the process. In the case of charged compounds, the tendency of diffusion is determined by both the concentration and electric potential gradient (electrochemical gradient), with the free energy obtaining the form [233]

$$\Delta G = RT \ln \frac{C_i}{C_o} + zF \Delta E_m \tag{2}$$

where $z$ is the solute charge, $F$ is the Faraday constant and $\Delta E_m$ is the potential differences in volts between the two compartments. The total difference in the free energy depends on the interplay between concentration and electric potential differences. For example, the efflux of K$^+$ is hindered by the electrical gradient despite the higher concentration gradient inside the cell [233].

5.3. Active carrier-mediated pathway
In some cases, certain compounds (usually relatively small hydrophilic molecules [8, 218, 234–238]) can bind to specific membrane protein carriers (e.g. GLUT1, which is a 55 kDa glucose transporter and provides the
main energy source for the brain [67, 236, 239–241] triggering a conformational change (CMT tends to be size and stereo selective [45, 78, 101, 144, 194, 242, 243]). As a result, the carriers, which can be found in different concentrations on both apical and basolateral membranes and are usually polarised, can transport the solute via diffusion along or against (at the expense of ATP) the concentration gradient (figure 2) [47, 59, 63, 78, 101, 195, 236, 244]. However, the direction of the concentration gradient usually favours transport from blood to brain [47, 194]. The use of carrier proteins as CNS drug delivery vectors is a possible route to delivering substances to the brain. In this case, one may also take into account the structural binding requirements of the transporter and the compound and the successful carrying of the solute from the luminal to the ablumenal surface [63].

5.4. Receptor-mediated transcytosis
This pathway is an active transport route that depends on temperature and can be saturated [101, 245]. RMT starts with an endocytotic event. In the case of RMT, cells have endocytotic receptors for many different types of ligands, e.g., hormones, growth factors, enzymes, lipoproteins and others [1, 6, 64, 86, 246–258]. Then, the transcytosis proceeds with the formation of a smooth plasmalemmal vesicle that consists of cholesterol and sphingolipid-rich rafts [259], which apart from the receptor and the ligand, also, contain extracellular fluid (in addition to vesicle-associated membrane protein-2 and other proteins [47, 199]), and are usually 50–100 nm in diameter. For success transcytosis within the cell, the vesicle should avoid the fusion with a lysosomal compartment that can create a secondary lysosome [6, 256]. In addition, the ligand may bind to a second intracellular receptor or follow another intracellular pathway that may include trafficking of endosomes and separation in the Golgi complex. Then, the separated ligand may be exported in the form of vesicles to a lysosome [1, 250]. The last step of RMT is the exocytosis of the receptor-ligand complex [260].

In summary, RMT is a very important pathway of delivering chemical agents to the brain by targeting particular receptors [8, 59, 63, 190, 261]. It is independent of various physicochemical properties (e.g. lipophilicity of the compound) and, also, independent of compound’s size, as well as other parameters. Moreover, it is considered safe due to its high specificity and the use of an entirely physiological mechanism [101].

5.5. Adsorptive-mediated transcytosis
Adsorptive-mediated transport is initiated through an electrostatic interaction between a positively charged compound (e.g. charged moiety of a peptide, ‘cationic’ proteins such as protamine and histone) [1, 63, 101, 262–265], and the negatively charged plasma membrane, due to the presence of glycocalyx and phospholipid head groups of the plasma membrane [266]. The advantage of AMT with respect to RMT is that it does not require specific binding to a receptor, but it is triggered by electrostatic interactions [78, 116, 248]. The process following endocytosis is similar to the RMT with vesicle formation, but differences on the affinity and capacity of these two mechanisms exist. Both AMT and RMT can be bidirectional, involve vesicles formation, and be both transcytosis mechanisms [67, 267]. AMT is generally very low at the BBB effectively blocking the translocation of serum proteins to the brain under physiological conditions, but it is still the preferred pathway for selective transport of plasma macromolecules (e.g. albumin and low density lipoproteins) to the brain [8, 9, 41, 67, 268].

5.6. Efflux transport pathway
Efflux transport is also an active carrier-mediated system by translocating distinct substrates from any point in the cell back to the systemic circulation in order to prevent the accumulation of compounds in the CNS [1, 54, 101, 269, 270]. This mechanism is very important, for example, for extruding pharmacological agents from the brain, which is the reason that many drugs fail to deliver to the brain [59, 271]. A common transporter example for this group is the ABC (ATP binding cassette) P-glycoprotein (P-gp) transporter, which is expressed on the luminal membrane of the ECs [67, 101, 122, 239, 269, 272–274]. In particular, P-gp prevents the passage of drugs and toxins (e.g. ingested toxic lipoprotein metabolites) into the brain facilitating their transport to the blood at the expense of ATP [47, 78, 269, 274]. Other examples, include the multidrug resistance transporters, monocarboxylate transporters and organic anion transporters, and organic anion transporter polypeptides [101, 269, 271, 275]. The latter transporters are unable to hydrolyze ATP. Hence, they are energy independent and cannot transfer compounds against their concentration gradient, which makes them bidirectional transporters that rely on concentration on both sides of the BBB [101].

5.7. Mononuclear cell migration
Mononuclear cells can enter the BBB through a diapedesis process without TJ’s disruption [59, 224, 276–280]. In the case of mononuclear cell migration, the ablumenal membrane opens after the closing of the luminal membrane during the process, in this way avoiding the formation of fluid-filled channels across the cell [6, 277]. In fact, the amount of translocated substances via this route is very small [281, 282], as may be suggested by the lack of leukocyte adhesion molecules on the lumenal surface and the small presence of immune cells in the parenchyma [67, 283]. However, in the case of neuro-inflammatory situations (e.g. stroke, multiple sclerosis), T cells, B cells, neutrophils, and macrophages are able to migrate into the CNS at sites of active lesions [284–287]. One way of transporting antibodies, erythropoietin and modified lysosomal enzymes to the brain can be achieved by extracellular
pathways allowing the substances to reach the Virchow–Robin spaces, which may be the case for compounds with long circulating half-lives and small volumes of distribution in the blood [49, 288, 289]. However, these mechanisms are poorly understood despite the fact that they may apply virtually for any kind of molecules. A notable example is the delivery of drugs through the ‘Trojan horse’ model [59, 278, 290–292].

6. Computational approaches

Some of the computational approaches for predicting the permeability of chemical substances at the BBB are based on the molecular properties of these compounds, such as MW, polar surface area, lipophilicity, hydrogen bonding capacity, charge, molecular size, shape and flexibility, etc [293]. The prediction is made based on the known values of logBB (the concentration of drug in the brain divided by concentration in the blood) and logPS (permeability surface area product) for the permeability of certain studied compounds [293]. In drug design particular properties are required, which are known as ADME standing for absorption, distribution, metabolism and excretion [294]. Lipinski’s rule of five, which are generally applicable for biological membranes and are also known as the Pfizer’s rule of five or simply the rule of five (RO5), provide a general framework for substances that will likely be biologically and pharmacologically active [295]. According to these rules, small lipophilic substances with MW less than 500 Da, number of bond donors less or equal than five and hydrogen bond acceptors less or equal than ten, and octanol-water partition coefficient \( \log P_{ow} < 5 \), will likely cross a biological membrane, which may also happen in the case of the BBB [294, 295]. There are exceptions to these rules, but these rules provide a qualitative measure [294]. The values of such physicochemical properties can be used as a training set of compounds and a test set (e.g. Kohonen’s self-organising map [296, 297]) together with statistical relationships derived empirically from experimental permeability measurements in quantitative structure–permeability relationship (QSPR) methods (or mentioned also above as SAR models) [294, 298, 299]. Common choice of QSPR descriptors include MW, polar surface area (PSA), partition coefficients, and hydrogen bond counts, which are known to correlate well with rates of permeation [294]. There are various empirical equations for different parameters, for example the Kety–Renkin–Crone (KRC) and Michaelis–Menten equations of capillary physiology that connects the permeability surface with the cerebral blood flow and other parameters [3, 300–302]. Although one can study passive membrane permeability with all-atom simulation models with explicit permeant and lipid molecules (e.g. using molecular dynamics simulations) [294, 303–311], such approach is computationally demanding. For this reason, QSPR models have included membrane-interaction descriptors obtained from molecular dynamics (MD) simulations of permeants in lipid layers [294, 312–316]. Coarse-grained (CG) models [317–320] and implicit membrane models [294, 321–324] have been used in order to enhance the computational efficiency.

Molecular mechanics models are particularly suited to studying passive membrane permeability, and compare well with experimental data and in silico predictions [294]. These models offer great advantages over others that require a ‘trained’ data set (e.g. QSPR). The main strategy to determine permeation rates is by measuring the conformational dependent free energy of dissolution for a molecule, which has shown very good agreement with experimental data. Taking into account deionization and estimates of entropy losses for the ligand and the membrane may improve the agreement with experimental data, which are based on the logBB (steady-state concentration of a drug in the brain over concentration in the blood) and logPS (permeability surface area product), where the permeability \( P \) can be found if the vessel surface area \( S \) can be estimated [4, 239, 294, 325]. Moreover, MD simulations based on a CG force-field have also been recently used to explore the possibility of opening the TJs due to shock wave induced bubble collapse, a method which has been used in experiments to enhance the permeability of drugs through the BBB [326]. This work has underlined the potential of using MD simulations of CG models to address physical aspects of the BBB.

MD simulations have been also combined with mathematical modelling recently to study the translocation of magnetic nanoparticles (NPs) across the BBB [327]. The success rate for this crossing is about 2.5% for nanometre-sized particles, whereas for micrometre-sized particles is about 0.1%. Here, the force profiles of the particle traversing the BBB in constant velocity were obtained. In another approach, steered molecular dynamics (SMD) simulations [328] for the BBB permeation were employed and mathematical modeling of the BBB as an input–output system has been considered [327]. From this model, the force profile required to overcome the barrier has been extracted for a single NP from the SMD simulations at a range of velocities [327]. Using these data a transfer function model was obtained and the diffusion coefficient through the barrier was evaluated. This study is a novel approach to bridge the gap between nanoscale and microscale models for the BBB highlighting the potential of multi-scale computational methods [327]. It also provides further possibilities for designed liposomes, which are used as ‘Trojan horse’ to carry substances into the brain [101]. Finally, MD simulations based on all-atom models and the MARTINI force-field [307, 329] have been used for the study of the membrane driven cis interactions of claudin-5 proteins in the formation of the BBB TJs [330]. The use of CG potentials with almost atomic resolution allow for long time scale simulations showing for seven different lipid compositions the formations of cis dimers and the subsequent aggregation into strands...
This study was able to capture the fundamental aspects responsible for the BBB TJs assembly offering the possibility of extending this framework on other TJs [330]. In this regard, the GomARTINI approach, which allows for the study of large conformational changes of proteins by using the MARTINI force-field, may enable further progress in this field [331].

Mathematical modelling has also considered the transport of water and solutes across the BBB (figure 3) [196, 332–334]. In this model, fluid flow in the cleft regions of the BBB was approximated by Poiseuille flow, while those in the endothelial surface glyocalyx layer (SGL) and BM were approximated by the Darcy and Brinkman flows, respectively [196]. This model was based on diffusion equations, which were solved. Anatomical parameters were obtained by electron microscopy studies, while various other parameters can be taken from in vitro and in vivo models [196]. The model has been able to predict that the BM and astrocytes of the BBB provide a great protection to the CNS under both physiological and pathological conditions [196]. Moreover, this study has shown that the astrocyte foot processes do not provide a significant resistance to water and solute transport across the BBB [196]. Numerical modelling could certainly offer more insight in the dynamic equilibrium of the BBB under certain modelling assumptions combined with experimental input.

Solubility-diffusion theory has been an additional means to predict the permeability based on three components: the partitioning between water and the membrane, the diffusion across the membrane, and the width of the membrane [294, 335–342]. This theory was further extended to include the size selectivity and chain ordering of lipids [294, 343]. A similar solubility-diffusion model has been employed for the BBB permeation using parameters including the air/water partition coefficient and molecular shape of the permeant, where also a Stokes–Einstein relation can be applied to describe diffusion across the BBB [294, 344, 345]. Such approaches, which may be computationally more demanding than QSPR models, may capture better the underlying physics of the permeation process and do not require ‘training’ with permeability data, making them more general and transferable [294].

7. Insights and future directions

In the last decade, significant success has taken place in understanding the translocation mechanisms of the BBB and several new carriers have been identified, [8] but the physical aspects of this dynamic interface still remain largely unexplored. Computer simulations have provided significant understanding of the BBB with the focus being on transferring efficiently drugs to the brain. Numerous studies have been based on empirical relations that use 'trained' sets of experimental data (e.g. physicochemical descriptors) [64, 239, 346]. There have also been a few computational approaches based on transferable force-fields and numerical modeling, for example, to predict transporter properties [347–349], protein activity and toxicity [350]. However, the increasing computational capabilities and the use of advanced transferable CG models, multi-scale approaches and continuum-scale modeling in the light of understanding the BBB could provide significant insight in the way particular transporters behave, or, even understanding physically the function of the BBB as a dynamic and highly selective interface [329, 351–353]. To this end, studies on biomembranes are very relevant for the BBB, as the apical and basolateral membranes of ECs play a dominant role in the translocation mechanisms. It is now a matter of
considering all the relevant processes under the same concept of the BBB to target a better understanding of the BBB for the design of efficient therapies.

Coarse-grained models have provided so far significant insight [294, 307, 322, 326–329] by focusing mainly on the free energy penalty that particular molecules have to pay in order to cross the cell membranes of the BBB. For example, free energy methods can estimate the water-membrane partition coefficient of small drugs, as well as the assessment of their toxicity, in this way examining a variety of molecular species ranging, for example, from benzene to more-complex anesthetics [303, 354–356]. This is already an advancement with respect to empirical models that are only based on physicochemical descriptors [64, 239, 346] and the main challenge is to acquire knowledge of the BBB structure at a microscopic level. Irrespective of the latter, simulations of this type can target specific processes, for example a particular receptor on the membranes, study of the affinity of various substances to the receptor and the subsequent endocytosis event. The MARTINI force-field [329] has provided a good basis for this kind of simulations. Especially for the case of proteins, the recent development of the GoMARTINI model allows for the study of large, particularly for the case of proteins, the recent development of the GoMARTINI model allows for the study of large


cosmological simulations are particularly suitable to providing understanding of the BBB by using numerical and continuum methods.

Multi-scale modeling can offer further opportunities for the study of the BBB. Recently, Smith et al have developed a hybrid scheme (CPL library) that combines MD simulations of CG models with computational fluid dynamics methods [353]. This method can still describe microscopic effects based on a CG description, while the computational fluid dynamics domains can include a number of different empirical relations, for example based on the physicochemical descriptors [64, 239, 346]. Further coarse-graining can additionally relieve the computational burden of simulating the BBB interface by considering implicit membrane models, as in the case of Feig et al [322, 362–365], which could be extended to the case of two implicit membranes surrounding a ‘cell’ medium. Larger time and length scales can be addressed by numerical modeling as has been shown in the case of Li et al [196], which provides a very good basis for studying the BBB by using numerical and continuum methods [196].

8. Concluding remarks

This review has presented the key features of the BBB required for understanding this dynamic, complex and adaptable interface. We mainly focused on the morphological and physical characteristics of the BBB, as well as on the translocation mechanisms of the BBB, by which substances are transported between the blood and the brain. We have also discussed computational approaches that have been used so far to investigate various features of the BBB ranging from computational methods based on empirical methods to molecular dynamics simulations of CG models and numerical and continuum approaches. We hope that this review will provide all the necessary information for inspiring multi-disciplinary research (in particular, research based on computational methods) with the aim of designing efficient drug therapies for the cure of CNS disorders, such as Alzheimer’s disease.

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