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CURRENT PERSPECTIVES

Current Chemical, Biological, and Physiological Views in the Development of Successful Brain-Targeted Pharmaceutics

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Abstract

One of the greatest challenges with successful pharmaceutical treatments of central nervous system (CNS) diseases is the delivery of drugs into their target sites with appropriate concentrations. For example, the physically tight blood–brain barrier (BBB) effectively blocks compounds from penetrating into the brain, also by the action of metabolizing enzymes and efflux transport mechanisms. However, many endogenous compounds, including both smaller compounds and macromolecules, like amino acids, sugars, vitamins, nucleosides, hormones, steroids, and electrolytes, have their peculiar internalization routes across the BBB. These delivery mechanisms, namely carrier-mediated transport and receptor-mediated transcytosis have been utilized to some extent in brain-targeted drug development. The incomplete knowledge of the BBB and the smaller than a desirable number of chemical tools have hindered the development of successful brain-targeted pharmaceutics. This review discusses the recent advancements achieved in the field from the point of medicinal chemistry view and discusses how brain drug delivery can be improved in the future.

Keywords Brain-blood barrier · Prodrug approach · Carrier-mediated transport · Receptor-mediated transport · Drug delivery

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Introduction

According to data from the World Health Organization (WHO), brain diseases account for almost 35% of all human diseases. The mechanisms of cellular exchange between the brain, blood, and cerebrospinal fluid protect the brain from harmful substances. However, they also represent a significant obstacle to the delivery of drugs and the treatment of many brain diseases [1]. Some therapeutics including antibiotics, antineoplastic drugs, or neuropeptides have been rendered ineffective in the central nervous system (CNS) diseases because of their inability to effectively cross the blood–brain barrier (BBB) and get delivered to the brain tissue [2]. To meet the challenges related to the functioning of the BBB and the delivery of drugs to the CNS, scientists are focusing their work on a comprehensive understanding of the barrier's mechanisms of action. A series of studies were carried out to compare the functioning of normal and disturbed BBB, and the major factors affecting this mechanism have been identified. Another important and rapidly growing area of research is the development of various approaches or methods of targeted drug delivery to the brain to treat CNS diseases such as Alzheimer's disease (AD), multiple sclerosis (MS), neuro-AIDS (acquired immunodeficiency syndrome), neuroinflammation, or cancer [3].

This review discusses the recent developments in the systemic delivery of therapeutics to the CNS. Targeted drug delivery may be achieved using chemical, biological, and physiological approaches that provide effective concentrations of the drug at the pathophysiologically relevant sites. Representative examples of the brain-enhanced delivery of small drug molecules, neurotransmitters, peptides, and genetic material are presented in detail.

General Background on the BBB and BCSF

Two main barriers determine the selectivity of transport within the brain (Fig. 1). Besides the well-known BBB, which consists mainly of the endothelium of the cerebral capillaries, there is also the blood-cerebrospinal-fluid barrier (BCSFB) composed of the epithelium of the choroid plexuses. Both barriers are similar in structure and therefore in some literature, the term BBB is used to refer to the final exchange by both cell barriers.

Another barrier in the CNS, apart from well-known BBB and BCSFB, is the blood-arachnoid barrier (BAB), which forms an essential interface between blood and cerebrospinal fluid (CSF) in the subarachnoid space (Fig. 1). The BAB is a multi-layered epithelium with tight junctions between cells of the outer continuous 1–3 cellular layers and makes up an effective seal covering the inner dural surface [4]. The pia and the inner layer of the arachnoid comprise one cell type — the leptomeningeal cell, which covers the outer layer of nervous tissue of the brain, the glia limitans [5].

The BBB instead is made up of a monolayer of polarized endothelial cells (EC), expressing glycosaminoglycans integrated with proteins and lipids of the cell membrane, and membrane receptors and enzymes. The capillary wall of endothelium has inwardly directed transporters that deliver mainly glucose, amino acids, and free fatty acids (FFA) to neighboring neurons [5].

The intercellular areas of the capillary endothelium are covered with a dense network of high-resistance connections, among which complex tight junctions (TJ) are the most important (Fig. 2) [6, 7]. In the basal part, there are also adherence junctions (AJ). In addition, other components of the barrier connections in the brain are the cell adhesion proteins, known as Junction Adhesion Molecules (JAM-A, -B, -C, and -D). In the brain, the most common adhesion particle for leucocytes is type A, which, through its terminal end embedded in the cytoplasm, binds to the guanylate kinase residue of the occludin domain [8, 9]. Apart from membrane proteins, the composition of the barrier connections also includes cytosolic proteins such as ZO-1 [10].

Due to such a precise organization of connections between ECs, the endothelium forms a continuous layer, which on its surface is additionally covered by pericytes capable of phagocytosis, fused with the basal membrane. This membrane is an extracellular matrix, which includes type IV and V collagen, fibronectin, and laminin [11, 12]. The functionality of ECs in BBB is also controlled by astrocytes [13]. Astrocytes take part in maintaining homeostasis through water transport, free radical scavenging, nutrient uptake/excretion, and ion buffering [14]. Pericytes also form BBB and cover 20–30% of the capillary surface. These cells are involved in regulating cerebral blood flow, maintenance of the BBB, and control of vascular development and angiogenesis [15]. The complex nature of the BBB permeability is also influenced by the presence of high levels of efflux transport proteins including P-glycoproteins (P-gp) and Multidrug-Resistant Protein-1 (MRP-1), and the expression of metabolic enzymes [6, 16].

The integrity of the structure of the brain barrier allows it to perform many functions, the most important of which is the precise exchange of chemical compounds between the CNS and the circulatory system [17, 18]. In addition, it protects against the effects of neuroactive substances and toxins circulating in the blood and allows to supply neurons with functionally important substances, such as glucose or amino acids [10]. Importantly, tissue integrity, repair, and

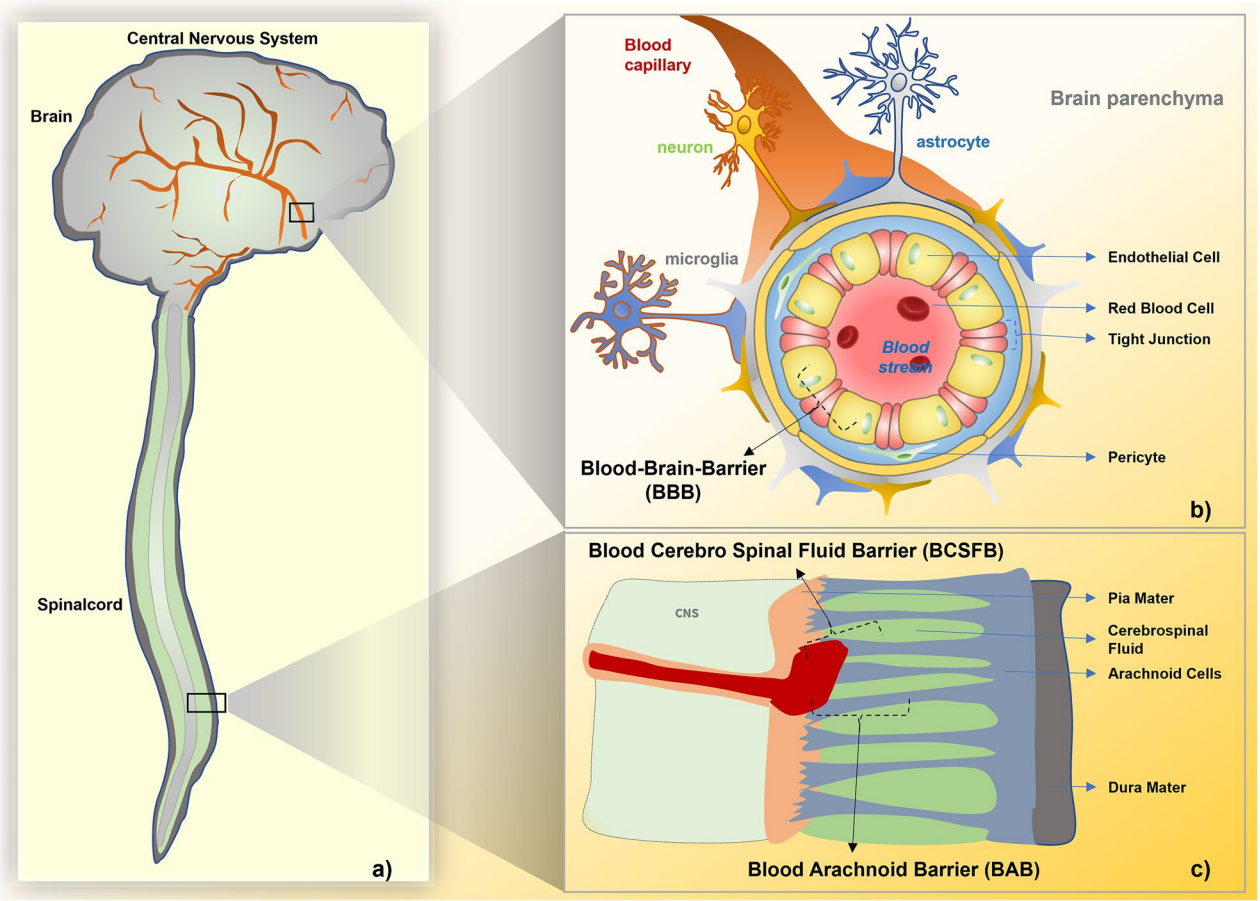

Fig. 1 Barriers of the brain substance delivery, **a**) the blood–brain barrier (BBB), **b**) blood-cerebrospinal-fluid barrier (BCSFB), and **c**) blood-arachnoid barrier (BAB)

homeostasis are also supported by the function of microglial cells, which are components of the CNS [19].

The blood-cerebrospinal fluid barrier (BCSFB) is functionally confined to the choroid plexus (CP) within the four brain ventricles, which plays a crucial role in the production of CSF. The choroid plexus consists of blood vessels, plexus parenchyma, and cubic epithelial cells. Capillaries, a component of the choroid plexus, differ in structure from other blood vessels of the CNS. Their endothelium has a window structure, which determines the movement of proteins and other components from the blood to the parenchyma [20, 21]. The outermost layer comprises polarized CP epithelial cells that are connected to the basement membrane and stromal layer on their basolateral side, allowing the cells to interact with systemic blood circulation. On the other hand, the apical-CSF-facing side produces CSF and exchanges materials with the ventricles [22]. Importantly, the BCSFB barrier secretes vitamin micronutrients and brain-modulating proteins: transthyretin and cystatin C protease inhibitor [23]. Although the BCSFB can be assigned certain anatomical structures, and the location, it is generally assessed in terms of functionality, not morphology. In functional terms, BCSFB includes a non-linear interaction between the diffusion of blood proteins into the CSF and its flow rate, which affects the final concentration of total protein in CSF. The exact functioning of BCSFB is therefore mainly determined by the CSF flow rate [23]. However, BBB is still the primary focus of scientists (54,200 records in PubMed in 2021 vs. 5,900 for BCSFB).

Brain Uptake Mechanisms—Transport Routes

The composition of the environment surrounding CNS neurons is subjected to precise regulation. Curiously, even the passage of plasma substances such as gases (O_2, CO_2, glucose, and amino acids), lipid-soluble compounds (ethanol, steroid hormones, thyroid hormones, and lipophilic drugs), or peptides weighing 400–800 Da to the CNS are selectively

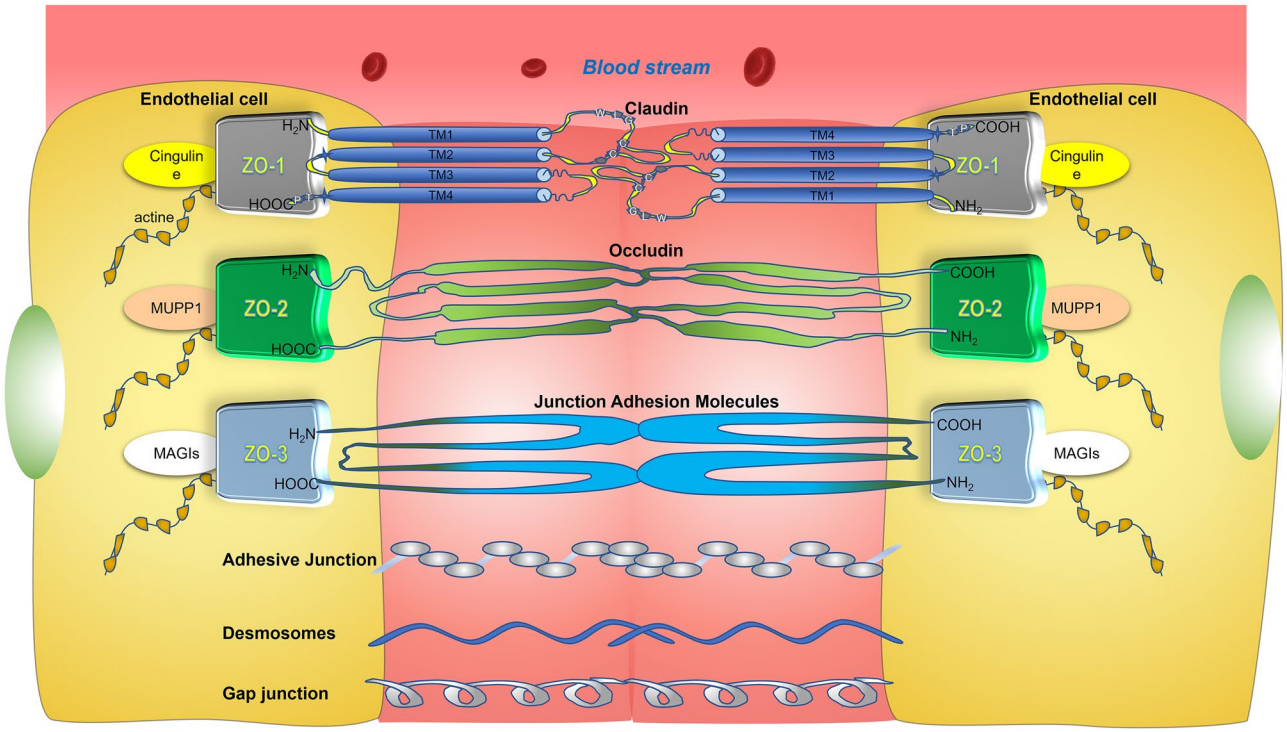

Fig. 2 Enlargement of the connections between the BBB endothelial cells

controlled by BBB activity (Fig. 3). Unfortunately, it has been generally accepted that the rate at which substances penetrate the brain tissue is inversely proportional to the size of the molecules and directly proportional to their lipid solubility [24]. Other important mechanisms by which molecules cross the BBB include saturable transport such as carrier-mediated or receptor-mediated transport, adsorptive endocytosis, and BBB disruption. Notably, the presence of

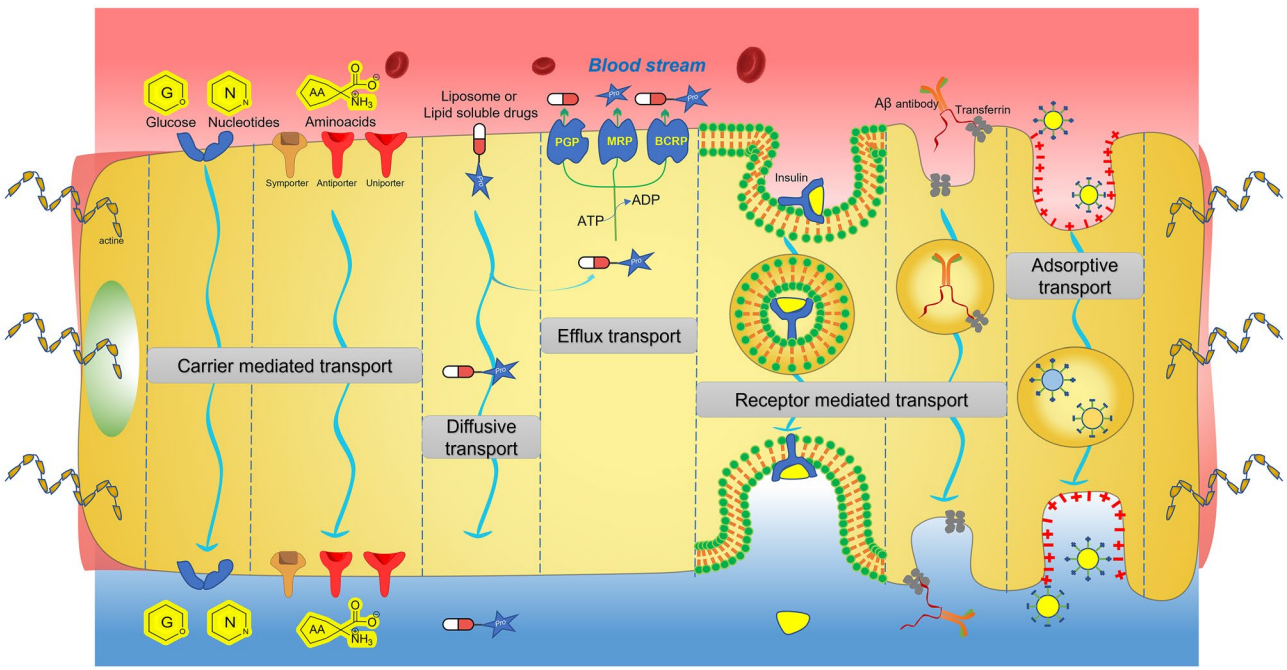

Fig. 3 Different pathways for substances to penetrate across the BBB endothelial cells

TJs between the adjacent ECs limits the diffusion of small molecules. Only water-soluble small molecules can diffuse through TJs in theory, but they do not diffuse in reality to any great extent [25, 26].

The solute carriers (SLC) constitute a superfamily of membrane transport proteins that facilitate the uptake of various compounds across the BBB. SLC transporters are classified as facilitated transporters or secondary active transporters because contrary to ATP-binding cassette (ABC) transporters, they do not need ATP for transport activity (Fig. 3, left side). The SLC carries solutes through electrochemical (Na^+ or H^+ gradient) or concentration gradients [27–29]. The spectrum of substrates for SLC transporters is very extensive and includes nutrients such as glucose, amino acids, nucleosides, monocarboxylates, and organic anions and cations. Importantly, some drugs (e.g., L-DOPA) are also transported across BBB by these transporters [30]. A large variety of transporters are present at the BBB, including a large neutral amino acid transporter type 1 (LAT1; SLC7 family), glucose transporter type 1 (GLUT1; SLC2 family), monocarboxylate lactate transporter type 1 (MCT1; SLC16 family), cationic amino acid transporter type 1 (CAT1; SLC7 family), choline transporter (ChT), concentrative nucleoside transporter type 2 (CNT2; SLC28 family), and sodium-coupled glucose transporters (SGLTs) [31]. The transporters are characterized towards their substrates by affinity, selectivity, stereoselectivity, and saturability [14]. The process of transport through BBB can be described kinetically. The researchers use the BBB permeability surface (PS) area, or the V_{max}/K_m ratio, where V_{max} is maximal transport capacity, and K_m is a substrate affinity for a transporter [14].

Ions pass through the barrier only by active transport. In the basal membranes of ECs, there is a sodium -potassium pump (ATPase Na^+/K^+) introducing sodium cations into the extracellular fluid of the brain and removing potassium from it [32]. An important role is also played by the Na^+/H^+ and Cl^-/HCO_3^- counter-transporters located in the luminal membrane [32]. Because of the interaction of these systems, Na^+ and Cl^- ions travel to the extracellular fluid, and K^+, H^+, and HCO_3^- ions in the opposite direction.

Larger molecules such as proteins, peptides, or lipoproteins are transported through the BBB by specific receptor and transcytosis mechanisms (Fig. 3, middle). Transferrin, lactoferrin, insulin, leptin, and lipoproteins are transported using receptor-mediated transcytosis (RMT). As reported before, this process is highly specific and requires endocytosis vesicles formation [33]. One of the best-recognized internalization pathways is clathrin-mediated endocytosis (CME), which takes part in transferrin, lipoproteins, and insulin transcytosis across the BBB [34]. In the first step of transcytosis, a ligand interacts with the receptor, and then clathrin-coated endocytic vesicles are produced. During this process, the membrane transforms into a vesicle of sizes between 70 and 150 nm. Another way of RTM is the caveolae pathway, which is used by low-density lipoproteins to cross BBB. As reported by Candela et al. [35], caveolae are 50–100 nm flask-shape vesicles built by caveolin-1 at the ECs. The main receptor systems taking part in the RMT through the BBB include transferrin receptor (TfR), insulin receptor (IR), and low-density lipoprotein receptor (LDLR).

The transferrin receptor is a unique receptor and is only expressed on the luminal side of ECs of the brain capillaries and not on other ECs in different tissues. TfR handles the transport of iron into the brain parenchyma to maintain iron homeostasis and thus is of great importance for proper brain function [36]. Over the past decade, TfR has become of interest to scientists, mainly in its use as a target to deliver drugs to the brain, which will be discussed in the following section [37]. IR, responsible for the transport of insulin into brain tissue, has also been a target of many in vitro and in vivo studies whose objective is to deliver drugs to the brain [37]. However, the results of the in vivo studies carried out by Rhea et al. [38] show that the signaling-related IR may not be solely responsible for the transport of insulin across the BBB. Apart from IR, the mannose 6-phosphate (M6P) receptor, also known as the insulin-like growth factor II (IGF-II) receptor, has also been examined as a receptor for transcytosis across the BBB [39]. LDLR, expressed in the apical membrane of ECs, recognizes LDLs and supports their endocytosis. Because of their localization, LDLR takes part in the uptake of lipoproteins and LDLR-related proteins 1 and 2 (LRP1, LRP2) from the circulation through RMT [37, 40]. An analysis of the current literature shows that lipoproteins have also been used to target LDLR for effective brain delivery [41]. A few other receptors have also been identified, such as neonatal Fc receptor (FcRn), leptin receptor (LepR) [40, 42], diphtheria toxin receptor, or type 1 scavenger receptor (SR-V1) [43].

Alternative transport for large molecules across the BBB is the adsorptive-mediated pathway (AMT), which does not require any interaction with the receptor (Fig. 3, right side). AMT, also known as pinocytosis, is mediated by the negatively charged surface layer of the cell membrane (glycocalyx), which enables the unspecific binding of cationic molecules that are then endocytosed to be trafficked across the ECs [33, 44]. This way of transport is used by polycationic peptides, which interact with the negatively charged head groups of a membrane. Similar to RMT, a vesicle is then formed around it, and the peptide is delivered to the other side of the BBB [45]. The examples of compounds that use AMT in the BBB are as follows, avidin, histone, and protamine [46]. Cell-mediated transcytosis is a more recently recognized route of transport across the BBB, which utilizes immune cells such as monocytes or macrophages to cross the intact BBB. According to Chen and Liu [25],

cell-mediated transcytosis is a unique transport route, which can be used virtually for the delivery of any type of molecules or materials.

BBB is also a structure, in which efflux transporters are present. Their main role is to eliminate the xenobiotic molecules. The current research shows that the inhibition of the activity of these transporters may be a promising manner, allowing for maintaining relevant drug concentrations in the brain. The basic function of efflux transporters, localized on the luminal and abluminal sides of ECs, is to remove metabolites and catabolites produced in the brain. The best-characterized efflux pumps in BBB are transmembrane P-glycoprotein (P-gp) and MRP1–5, which belong to energy-dependent ATP-binding cassette (ABC) transporters. Importantly, modulation of function of efflux transporters, such as P-gp and MRP1-5 can lead to increased drug concentrations in the brain. However, this process can also result in significant CNS toxicity [14, 47].

Methods to Assess BBB Penetration

Determination of the drug's ability to cross the BBB is one of the most important aspects in the discovery of novel CNS therapeutics. The screening strategies for brain penetration can be divided into in vitro and in vivo methods. In vitro methods, including the parallel artificial membrane permeability assay (PAMPA), cell-based monolayer transport assay is accurate and suitable for screening a large number of drug candidates [48]. In turn, commonly used in vivo methods include the determination of drug concentration in brain and plasma (C_{brain}/C_{plasma}), microdialysis, and brain exposure assessment (BEA), method for evaluation compound CNS distribution using $K_{p,uu}$ (unbound brain-to-plasma partition ratio) as the index [48].

The assessment of drug concentration in the brain and plasma (C_{brain}/C_{plasma}) is one of the most frequently used methods to determine brain distribution. In this strategy, blood and brain samples are collected at certain time points after drug administration, and the concentrations are calculated using LC–MS/MS technique [49]. The ratio C_{brain}/C_{plasma} below 0.1 is regarded as a profound limitation to crossing the BBB [50]. The free drug hypothesis led to the increased interest in the unbound drug concentration (C_u) as the active species that exerts pharmacological effects in the brain. Another term related to this theory is the fraction unbound (f_u), which defines the free drug available in tissues [51]. Other terms have also been introduced into use. They are as follow, the unbound drug concentration in the plasma [$C_{u,p}$ = total drug concentration in the plasma (C_p) × $f_{u,p}$] and in the brain [$C_{u,b}$ = total drug concentration in the brain (C_b) × $f_{u,b}$] as well as the corresponding unbound brain-to-plasma partition ratio ($K_{p,uu} = AUC_{u,b}/AUC_{u,p}$). The value of the last parameter, $K_{p,uu}$, is currently regarded as an indicator of brain permeability in vivo [48, 52]. Importantly, $C_{u,b}$ and $C_{u,p}$ can be affected by efflux transporter status and passive permeability of a drug [49]. $C_{u,b}$ is comparable to $C_{u,p}$ for highly permeable drugs which are non-efflux substrates because distribution equilibrium can be reached quickly among the blood, the brain, and the CSF compartment. On the other hand, $C_{u,b}$ is much lower than $C_{u,p}$ for therapeutics being efflux substrates or exhibiting poor permeability [49]. For example, $C_{u,p}$ was > 20-fold higher than $C_{u,b}$ for loperamide, amprenavir, benzylpenicillin, cimetidine, and sulpiride after intravenous infusion to rats [53]. These results were further confirmed in studies using the Mdr1a(-/-) rat model in which the scientists reported increased brain exposure to the drugs [53]. When assessing the ability to permeate to brain consideration must be given to species differences in transporter expression and functional activities. Furthermore, pathophysiological conditions such as aging, AD, PD, or multiple sclerosis have been described to affect BBB integrity, permeability, and transporter activity [50]. The need to assess drug concentrations in brain and plasma, and its free fractions is highly vital in the case of brain tumors treatment [52]. For instance, it was found that in human epidermal growth factor receptor 2–positive breast cancer brain metastases, lapatinib concentration varied from 21 to 700% of serum concentrations [54]. Without achieving adequate free drug concentration in the brain, it is impossible to conduct effective anti-neoplastic treatment [52].

Chemical Approach—Drug Manipulation

In general, there are many ways to increase drug delivery into the CNS. The most often applied approach is based on the chemical modification of the pharmacologically active compounds to improve their physicochemical characteristics (Fig. 4) [55]. Because most drugs in the circulatory system are bound to proteins (e.g. albumin or alpha-acid glycoprotein), only the unbound drug in the aqueous phase is available to cross the BBB. The free drug molecule then crosses the lipophilic membrane of the ECs of the BBB. A few physicochemical properties of compounds are involved in this process: drug lipophilicity, its ionization, molecular size, hydrogen bonding, polar surface area, and affinity towards plasma proteins [55].

Physicochemical Characteristics

The relative affinity between water and lipid, expressed by its octanol/water partition coefficient (logP), is the most frequently described parameter determining the ease by which the molecule can cross the lipid membranes of the ECs. The suggested range for logP parameter is between 2 and 4 [48];

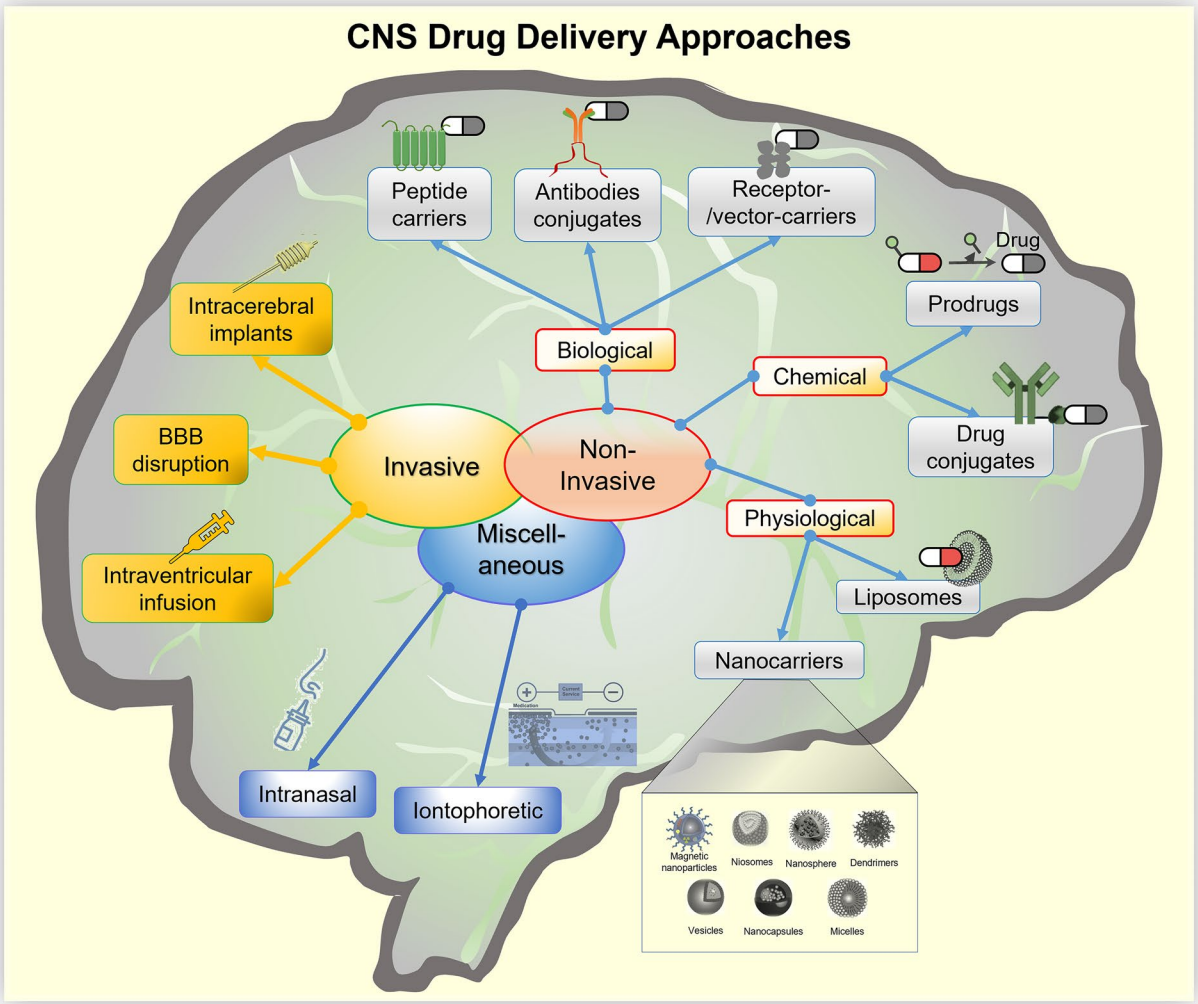

Fig. 4 Different approaches improving the CNS drug delivery

however, some authors indicate the ideal logP range for BBB permeability to be 1.5–2 [14]. It also should be highlighted that this parameter depends on the molecular weight of the drug, its basicity, and pK_b.

The ionization of the drug molecule at physiological pH is an adverse factor in terms of ECs permeation because the ionized compounds are surrounded by water molecules, which disfavors membrane interaction. The net movement of a compound with anionic charge, similar to EC membrane, is thermodynamically unfavorable, due to mutual repulsion of negative electrostatic charge [14]. The effects of lipophilicity on the ability to cross the BBB were the subject of many reviews in the past [14, 56]. Therefore, in this work, we focussed on other factors that improve drug penetration through the BBB. Molecular size is of importance for the diffusion rates of drug molecules. There is a linear relationship between permeability and $P/\sqrt{Mw}$ (P: partition coefficient octanol/water, square root of molecular weight). This relationship is restricted to molecules with $Mw < 1000$ Da [57]. The effect of plasma protein binding on the transport across the BBB is unequivocal. For a long time, it was believed that only free drug molecules could cross the BBB. However, some reports have demonstrated a significantly greater brain uptake of several protein-bound hormones and drugs than expected from their free plasma concentration [58]. Based on these results it was hypothesized that in the brain microcirculation dissociation of the drug-protein complex is enhanced [55, 59].

One of the most frequently applied ways to increase the BBB permeation of the drug molecule is to synthesize its more lipophilic derivatives because lipophilicity is one of the most important parameters related to CNS penetration

[48]. The well-known example of this approach is that of heroin, a diacyl derivative of morphine, which crosses the BBB approximately 100 times more easily than its parent drug. Because of the direct correlation between lipophilicity and BBB permeability, hydrophobic precursors of hydrophilic drugs were thought to be more permeable. However, although the exact uptake mechanism of heroin across the BBB is still unknown, it has already been shown that morphine is a P-gp substrate and thus, effluxed out of the brain, while heroin is not, showing the P-gp may play a bigger role in BBB permeation of these compounds than the hypothesized lipophilicity-related passive diffusion [60]. Moreover, the results of this strategy have frequently been unsatisfactory mainly because of the changes in drug-like parameters and ADME processes, such as water solubility, metabolic half-life (t*1/2*), and clearance (CL) [33, 48, 56]. It happens frequently that lipidation of a polar parent drug contributes to the enhanced drug delivery to the brain but it does not correlate with an improved in vivo efficacy. It can be partially explained by the fact that increasing lipophilicity of the compound also increases its non-specific binding to brain tissue and thereby reduces its availability for its therapeutic target within the brain parenchyma [33].

For instance, the studies on the lipophilic analogs of nitrosourea showed that anti-cancer properties of the drug candidates were inversely proportional to their lipophilicity. It was explained by the fact that more lipophilic compounds present lower solubility in the aqueous plasma and easily bind to plasma proteins. This, in turn, leads to lower concentrations of drugs available for diffusion into the CNS [61]. This observation allows us to conclude that when a drug molecule is used to treat CNS diseases, an optimal balance between cerebrovascular permeability and plasma solubility is required [56]. The reverse strategy to increasing lipophilicity may be to remove the polar groups in a drug, however, this approach might lead to the decreased interaction of a molecule with a molecular target [62]. In the light of recent research results, it should also be considered that some of the CNS drugs act by reaching their targets by diffusion through cell membranes [63]. It has been found that some small-molecule drugs interact with their targets (receptors, ion channels, or transporters) which are located at the phospholipid bilayer of cellular membranes. It means that a drug must first partition in the phospholipid membrane before reaching the protein target. Therefore, the membrane access mechanism, and underlying ligand–lipid interactions strongly influence drug's activity, structure–activity relationships, pharmacokinetics and physicochemical properties [63].

Another method used for the improvement of the BBB permeation is the reduction of hydrogen bond donor capacity (HBD). Lipinski's rule of five (RO5) suggests the HBD of drug candidates as < 5; however, CNS drugs usually have HBDs less than three. The studies in medicinal chemistry showed that reducing the HBD capacity of a drug candidate can be a promising approach for improving BBB penetration [48]. This strategy was applied by Sakai et al. [64] who put 3-chloropyridazine in place of 5-aminopyrimidine in fibroblast growth factor (FGF) receptor modulator and obtained a greater total brain-to plasma partition ratio (*K*p).

Recent research is also focused on tPSA, a parameter describing the sum of surfaces of polar atoms in a drug candidate. The number of polar atoms in a molecule was found to determine membrane transport, BBB permeability, and drug metabolism [65]. Molecules with a PSA greater than 140 $Å^2$ poorly penetrate cell membranes, while those with PSA below 60 $Å^2$ are easily absorbed [65]. Therefore, reducing tPSA has become a promising strategy to improve the BBB targeting. This approach was applied by Rover et al. who found a correlation between tPSA and the BBB penetration in a series of kynurenine monooxygenase (KMO) inhibitors [66].

Enhancing rigidity appears to be another promising way to increase BBB penetration. An example would be a chemical modification of erlotinib, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, based on the closure of alkoxy chains and formation of 1,4-dioxane chain. This change resulted in tenfold increased BBB penetration [67]. Medicinal chemists proposed also reducing pK_a as a way to enhance the BBB permeation since the value of pKa correlates with the efflux ratio. For instance, reduction of basicity in molecules of FGF receptors modulators resulted in sixfold lower efflux ratio [64].

Nanocarriers as Devices Improving Physicochemical Properties of Drugs

Another method for increasing the BBB permeability of a hydrophilic drug is to incorporate the drug molecule into a sphere of lipids in a form of a liposome. This strategy allows for changing a matrix of a hydrophilic drug in which it is distributed. These nanocarriers comprise an aqueous core surrounded by a bilayer of lipid, mimicking the cell membrane. This structure helps in the fusion of liposomes with the cell membrane and subsequent uptake in the cells. Liposomes can transport effectively both hydrophilic and hydrophobic therapeutic agents [68]. However, even small unilamellar liposomes, do not cross the BBB substantially in the absence of vector-mediated drug delivery [56]. The next important drawback of liposomes is that they are rapidly removed from the bloodstream after intravenous administration, because of extensive uptake by cells lining the reticuloendothelial system [56]. The problem of limited BBB transport and rapid clearance of liposomes can be overcome by applying the PEGylation technique. Polyethylene glycol (PEG) of molecular weight below 6000 Da is biocompatible, non-immunogenic, and non-toxic and shows good solubility

in polar and non-polar solvents [69]. Importantly, liposomes increase drug stability, solubility, and prolong the circulation time. All these features make PEG a promising candidate in the design of CNS-drug delivery systems [70]. PEGylation of nanocarrier (e.g. liposome) was found to improve its biocompatibility and avoid the opsonisation of nanocarrier [68]. On the other hand, PEGylation isolates the targeting ligand and decreases the interaction with a specific target, which can contribute to lower interaction with the desired receptor site and reduced therapeutic outcome [68]. PEGylated liposomes, nanoparticles (NPs), and dendrimers are at the center of scientists' focus in CNS drug delivery. These devices can also be used as carriers of peptides, proteins, or genetic material. Below, we present just a few selected examples of research on PEGylated liposomes for drug delivery to the CNS. PEGylation technology can be used together with chimeric peptide technology to improve BBB transport and inhibit the peripheral clearance of liposomes [71]. Huwyler et al. [71] constructed PEGylated immunoliposomes (antibody-directed liposomes) containing a thiolated murine monoclonal antibody (Mab) and a 1,2-distearoylphosphatidylethanolamine (DSPE) moiety at the other end for incorporation into the liposome surface for the delivery of antineoplastic drug daunorubicin. These highly advanced immunoliposomes could cross BBB using receptor-mediated transport [71]. In turn, transferrin-conjugated PEGylated liposomes tethered with a glial fibrillary acidic protein promoter (pGFAP) or cytomegalovirus promoter (pCMV) were used as nanocarrier of brain-derived neurotrophic factor (BDNF), which has a vital function in restoring CNS damage. Both these liposomal formulations showed great potential to cross the BBB. In addition, with pGFAP liposomes, the expression level of BDNF in the cerebral cortex was substantially increased [72]. Several other examples [73–82] of PEGylation to transport drugs across the BBB are shown in Fig. 5.

Some studies indicate that cyclodextrins may improve drug delivery to the CNS; however, this field of science needs to be carefully and comprehensively examined [83, 84]. On the other hand, the review of the latest data allows for concluding that CDs might be particularly useful in the treatment of cardiovascular and CNS diseases due to their promising effects on cholesterol metabolism [85].

Prodrug Approach

Another often used chemistry-based strategy that has been successfully applied to improve the CNS transport of small therapeutic agents is their chemical modification by employing the prodrug approach [86]. Prodrugs are generally defined as bioreversible analogs of drug molecules, which should undergo chemical or enzymatic biotransformation to transform into an active form that exerts the proper biological effect (Fig. 6). The primary function of prodrug design is to overcome the multiple physicochemical, pharmaceutical, biopharmaceutical, or pharmacokinetic shortcomings of the parent molecule, which otherwise would limit its clinical

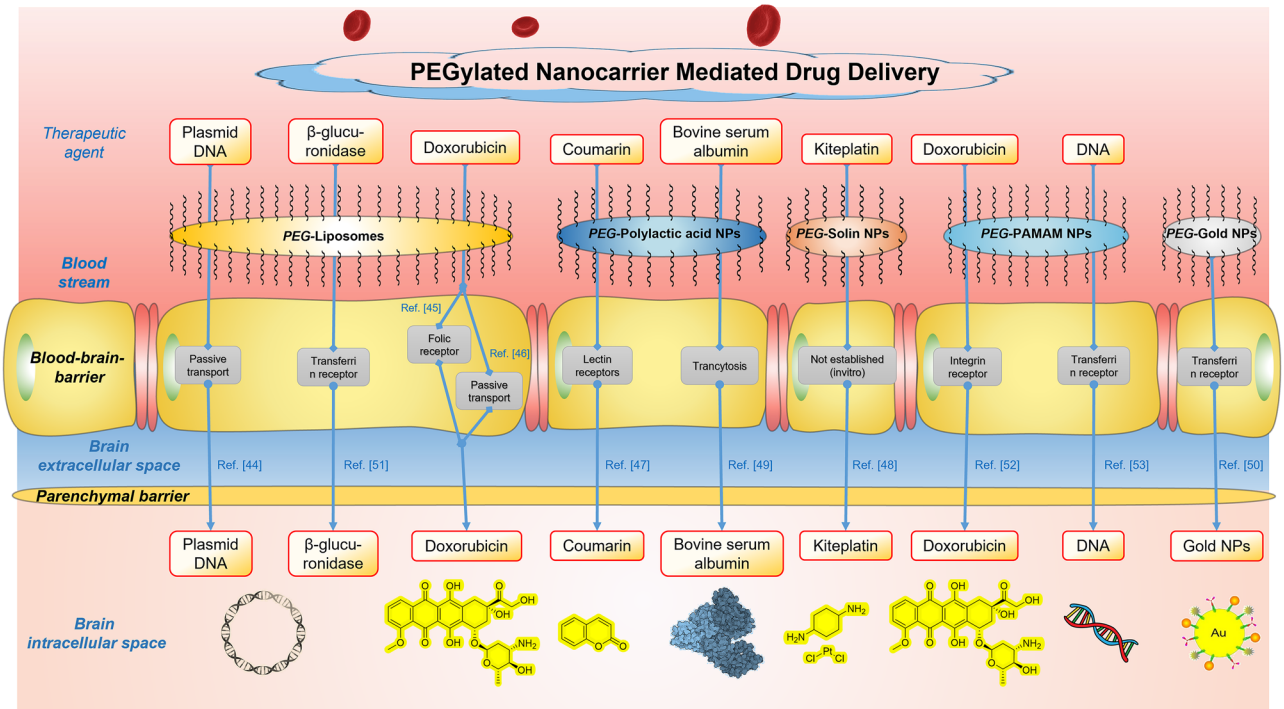

Fig. 5 Examples of PEGylated nanocarrier-mediated brain delivery of therapeutic agents

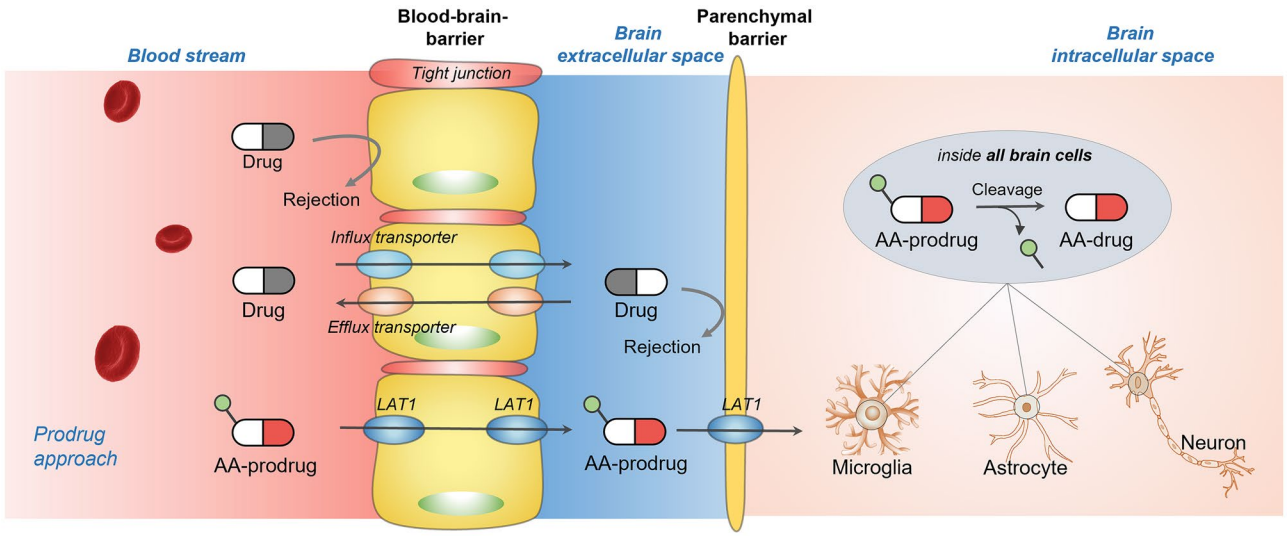

Fig. 6 Principle of transporter (L-type amino acid transporter 1, LAT1) -mediated prodrug approach, in which amino acid (AA) is attached to the parent drug to mimic LAT1-substrates, which is then cleaved in the brain parenchymal cells enzymatically

administration [86]. The professional pharmaceutical literature mentions the following applications of prodrugs, overcoming the drug formulation problems, improvement of pharmaceutical properties of drugs such as poor aqueous solubility, chemical or enzymatic instability, improvement of pharmacokinetic properties: inadequate oral absorption or distribution, rapid metabolism, low brain penetration, and decreasing toxicity or local irritation [87]. The prodrug approach appears also to be a very promising tool to improve targeting of drug action, particularly anti-cancer activity [87]. To improve a site-specific delivery, a suitable enzyme could be tagged with a monoclonal antibody so that they are incorporated at the target tissue. A prodrug, which is then administered, upon reaching the target tissue, is then converted to its active form. For instance, β-lactamase is used for the activation of vinca alkaloids [70].

A wide variety of promoieties have been used to solve the potential problems associated with parent drugs. The choice of promoiety is generally determined by the purpose of the prodrug, type of functional groups available on the parent drug, chemical and enzymatic conversion, the safety of the promoiety, and ease of pharmaceutical formulation [88].

One of the most frequently cited examples of prodrug modification to improve BBB delivery is morphine and its related compounds, codeine, and heroin. These both derivatives are formed through *O*-methylation and *O*-acetylation of morphine, which results in 10- and 100-fold increase in BBB transport, respectively. Codeine and heroin are converted to morphine in the brain, which interacts with the opioid receptor [89] (Fig. 7). As mentioned above, the prodrugs must be activated either by chemical or enzymatic means. BBB provides an adequate environment to convert prodrugs into drugs due to the presence of many enzymes, including esterases. One example of esterase utilization to convert a prodrug into an active molecule is acetorphan, which is the benzyl ester of *S*-acetylthiorphan, a derivative of thiorphan [90]. Thiorphan was discovered in the early 80 s of the previous century and is a specific enkephalinase inhibitor with antinociceptive activity, but incapable of crossing BBB [91]. Chemical modification into acetorphan leads to increased lipophilicity and improved brain delivery. Following CNS entry acetorphan is hydrolyzed by esterase to the more active inhibitor thiorphan [90]. Esterases can also be used as activating enzymes of ester-based prodrugs of non-steroidal anti-inflammatory drugs (NSAIDS). For instance, Yoshiharu et al. [92] reported synthesis of a triglyceride prodrug of ketoprofen (1,3-diacetyl-2-ketoprofen glyceride), which extremely weakly crosses BBB due to the complete ionization of its carboxyl group at physiological pH and moderate lipophilicity. The addition of diacetyl glyceride to the carboxylic group of ketoprofen improved lipophilicity and blocked the ionization of the carboxylic acid group. This modification resulted in greater transport through the BBB and more effective delivery of ketoprofen to the CNS. However, this strategy was not found very useful since ketoprofen was easily effluxed from the brain [92]. In turn, adenosine deaminase (ADA) was studied as an activation enzyme for the delivery of 2'-beta-fluoro-2',3'-dideoxyadenosine (F-ddA) and 2'-beta-fluoro-2',3'-dideoxyinosine (F-ddI), acid-stable analogs of dideoxyadenosine (ddA) and dideoxyinosine (ddI) [93] (Fig. 7). There are other enzymes such as xanthine oxidase, monoamine oxidase, and cytochrome-P450 enzymes, which can be utilized as biotransformation systems in the conversion of drugs that cannot cross BBB [89]. For

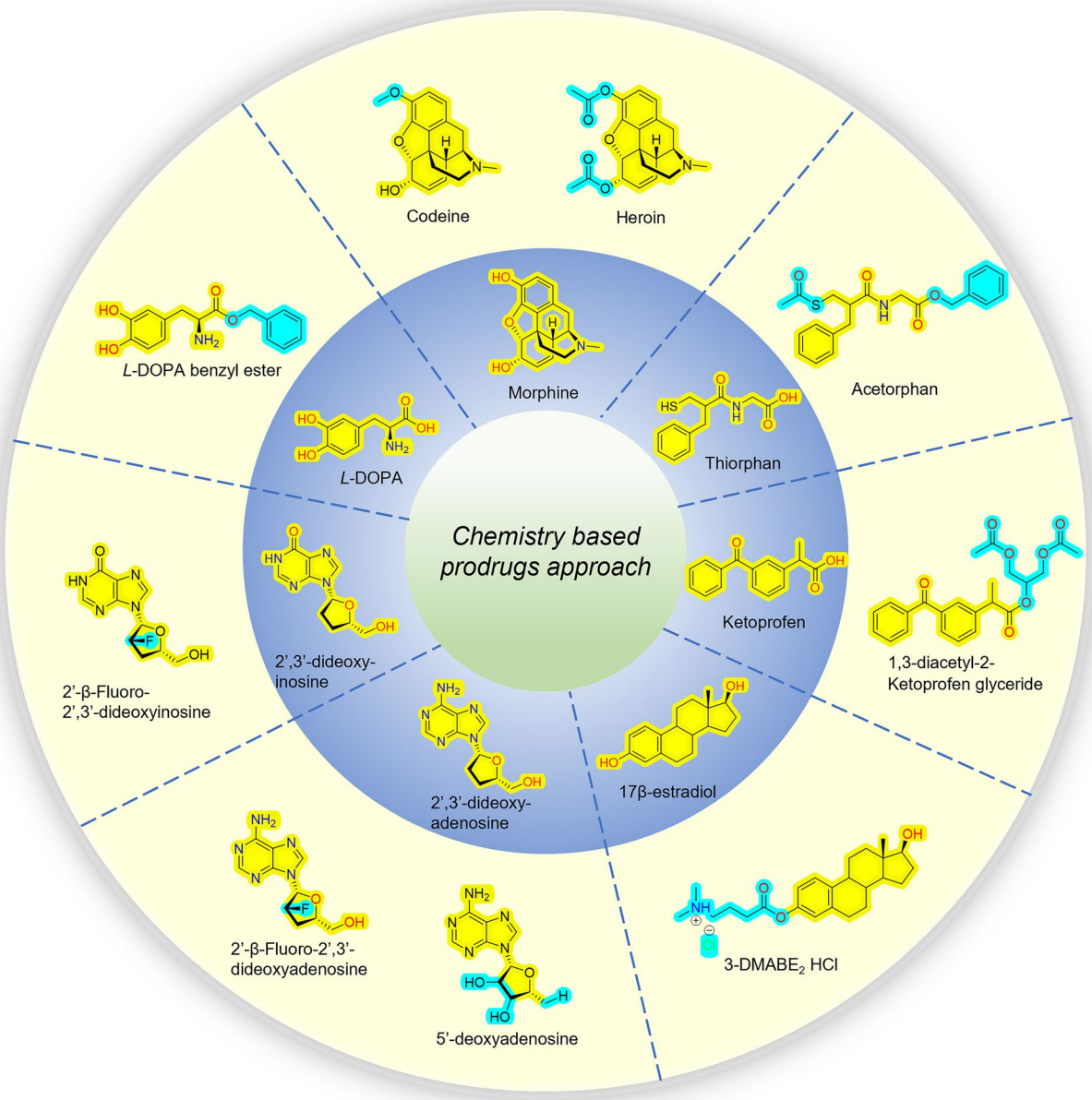

Fig. 7 Prodrug approach to target the BBB

example, xanthine oxidase was used as a biotransformation agent for enhanced brain delivery of an anti-HIV nucleoside 2'-F-ara-ddI (didanosine) [94].

The prodrug approach is often utilized in the targeted drug delivery through carrier-mediated transport. Carrier-mediated transport requires highly stereospecific substrates and presents specific structural requirements. As a result, therapeutic agents are not generally transported by carrier-mediated transport (CMT) systems, but prodrug strategy appears to be a good method to overcome these drawbacks [89]. Thus, the newly synthesized prodrugs are quite often made of the active parent drugs, which are conjugated with the promoieties that resemble specific substrates of certain transporters. Ideally, the drugs are released after enzymatic cleavage from their prodrugs after reaching brain tissue and these approaches have been successfully developed for the BBB transporters such as LAT1, GLUT1, and SVCT2 (Fig. 3, left side) [89].

A prodrug approach can be also utilized in brain-targeted nasal delivery. Appropriate chemical modification of drug molecules can change their chemical-physical properties (octanol/water partition coefficient, stability) and enhance their ability to cross the nasal mucosa. In addition, prodrug strategy enables to decrease metabolic degradation of the drug, and therefore, improves its bioavailability. As an example, Kao et al. [95] studied the nasal route for the systemic delivery of L-dopa using water-soluble ester prodrugs of L-dopa. It was found that water-soluble prodrugs for the nasal delivery of L-dopa to the CNS are characterized by advantageous features such as improved bioavailability, decreased side effects, and potentially enhanced CNS delivery [95]. Another example of a prodrug approach for the nasal delivery of drugs to the CNS might be ester prodrugs of 17β-estradiol, which allows obtaining high estradiol concentrations in CSF [96].

Chemical Drug Delivery Systems

The chemical drug delivery system (CDS) term was first introduced by Bodor and relates to a wide variety of possibilities for site-enhanced or site-specific delivery [97]. The CDS is produced by chemical reactions from the target drug, which is then covalently coupled with one or more carriers. In contrast to the prodrug approach, a CDS typically requires only a single activation step [98]. By design, after delivery, the CDS will undergo enzymatic conversion, which produces intermediates having different physical properties, thus ultimately allowing a preferential and favorable distribution of a parent drug at the site of action [97]. Literature review shows that CDS can allow not only the site-enhanced delivery but also the sustained release of pharmacologically active concentrations at the active sites. The issue of CDS has been extensively studied, and three types of CDS can be distinguished: (i) enzymatic physicochemical CDS, (ii) site-specific enzyme-activated CDS, and (iii) receptor-based CDS. For instance, 1,4-dihydro-*N*-methyl-nicotinic acid (dihydrotrigonelline) is a frequently used lipophilic target or moiety that can increase the brain delivery of a wide variety of drugs [98].

CDSs based on the redox conversion of a lipophilic dihydropyridine to an ionic, lipid-insoluble pyridinium salt, have been developed to improve the delivery of various types of drugs to the CNS. Generally, a dihydropyridinium-type CDS crosses the BBB by passive transport due to the high lipophilicity, then it undergoes enzymatic oxidation to an ionic pyridinium compound, which promotes retention in the CNS [99]. Redox CDS has been extensively applied to the brain-enhanced delivery of a wide variety of drugs, including steroid hormones, neurotransmitters, anticonvulsants, antibiotics, antiviral, and anticancer agents [99].

Molecular Packaging

The delivery of peptides to the CNS can be enhanced using "molecular packaging" method. In this method, the peptide unit makes up a part of a bulky molecule, in which the majority are the groups preventing recognition by peptidases and directing to BBB penetration [100]. This strategy allows for increased lipophilicity and concomitant improved passive transport, improved enzymatic stability, and brain targeting due to the lock-in mechanism. As an example, brain delivery of thyrotropin-releasing hormone has been improved using the molecular packaging strategy [101].

Biological Approach

Biological approaches of CNS drug delivery primarily originate from the understanding of the anatomical and physiological basics of transport through BBB. Conjugation of a drug with antibodies is one of the most widely known examples of the biological approach in targeted drug delivery to the CNS (Figs. 3 and 4). The antibody–drug conjugate is directed towards an antigen residing on or within the target tissues, in this case, the brain. Antibodies are particularly well suited for RMT systems in the BBB given their high affinity and specificity for their ligands. Other biological methods such as vector-mediated transport, molecular trojan horses, or chimeric peptides will also be briefly discussed in the following sections (Fig. 4).

Antibody–Drug Conjugates

The high specificity of monoclonal antibodies contributed to the increased scientific interest in their application in drug delivery to the CNS to treat Alzheimer's or Parkinson's disease (AD, PD). It has been established that only 0.05–0.1% of the initial dose of injected antibodies enter the brain [102]. For instance, Faresjo et al. [103] reported the mean brain concentrations for [^{125}I]mAb3D6-scFv8D3 and [^{125}I] di-scFv3D6-8D3b at 2 h post injection to be 1.1 ± 0.23%ID/g brain and 0.76 ± 0.06%ID/g brain, respectively. In addition, both antibodies had increased brain uptake compared to unmodified IgGs (ca. 0.03%ID/g brain). When assessing the concentration of antibodies in the brain parenchyma, it should be considered that this is an extremely difficult aspect to implement. Many researchers treat CSF as a surrogate of brain interstitial fluid (ISF) and estimate drug concentration in CSF. However, CSF to serum ratios for antibodies are usually higher than respective ISF to plasma ratios, and consequently, brain uptake of antibodies may be overestimated [104]. The efficacy and safety of immunotherapies used for CNS disorders could be improved through an increase in antibody brain penetration. The main mechanisms involved

in the transport of antibodies to the CNS include adsorptive-mediated endocytosis (AMT); carrier-mediated transport (CMT) and receptor-mediated transcytosis (RMT) [105, 106]. Indeed, the issue of antibody entry into the CNS is related to the pharmacokinetics of this process, which is not yet well understood [103]. This problem was tackled by Syvänen et al. [107] who assessed the brain distribution of antibodies designed for brain TfR1-mediated transcytosis. The authors reported that these antibodies enter the parenchyma mainly through the ECs of the BBB. Importantly, it was found that the studies reached the brain to a greater extent and were more uniformly distributed inside the brain compartment and parenchyma compared with unmodified IgG antibodies [107, 108]. The scientists reported also that the size of the antibody may affect its ability to diffuse in the cells of the brain parenchyma since brain distribution of a 15 kDa single domain antibody (sdAb) was greater than that of 150 kDa IgG antibody [109]. The issue of brain PK of antibodies was further studied by Faresjö et al. [103] reported that smaller size (58 kDa) [^{125}I]di-scFv3D6-8D3 antibody showed faster elimination from blood, lower brain C_{max}, and T_{max}, than larger size [^{125}I]mAb3D6-scFv8D3. However, the smaller antibody exhibited a larger parenchymal to capillary concentration ratio, and a net elimination from brain at an earlier time point after injection compared with the [^{125}I]mAb3D6-scFv8D3. Importantly, the authors did not find the differences between the elimination rate from brain between the antibodies [103]. Therefore, it might be concluded that the size of the antibody affects parenchymal delivery, but it does not influence the elimination.

There are several methods to improve the delivery of antibodies to CNS. For instance, cationization of antibodies by attachment of primary amine groups to their surface increases their uptake into the brain by AMT. Although the capacity of AMT is high, this mechanism is a process of low affinity and therefore is characterized by poor specificity. Improved transport through CMT can be achieved by coupling the antibodies with specific endogenous substrates such as glucose or amino acids. However, this process is very challenging for the transport of antibodies because GLUT1 or LAT1 transporters carry only small molecules and are highly stereoselective [110]. In turn, optimization of the affinity of the ligand that is targeting specific receptors in RMT is another effective strategy for improving the uptake of antibodies [111].

One strategy to improve BBB transport of antibodies is the development of BBB-crossing bi-specific antibodies, which have been engineered to incorporate one specificity against a BBB RMT receptor and the second against a CNS therapeutic target to produce a pharmacological effect. Another method can be the customized design of antibody constructs with physicochemical, molecular, and binding properties better optimized for successful transport across the BBB [105]. These methods are currently used for the brain-targeted delivery of monoclonal antibodies against β-amyloid to treat AD [112].

Peptide-Vector Strategy

A few small synthetic peptides such as pegelin have been developed to effectively cross the BBB and to transport conjugated therapeutic molecules [113]. However, little is known about the mechanism they used to cross the BBB and enter the brain. Some authors imply the peptides can worm their way directly through the cell membrane [114]. For instance, transactivating-transduction peptide (TAT) is involved in the replication of HIV by penetrating the nuclear membrane and acting as an activator of transcription [114]. Torchilin et al. [115] proposed the TAT peptide induces the formation of reverse micelles as an energy-independent process. Cell-penetrating peptides are built of an amphipathic α-helix and contain hydrophobic domains and positively charged domains. These domains comprise repeating sequences of a charged amino acid (e.g. arginine or lysine) followed by a series of hydrophobic residues [114]. Rousselle et al. [116] conjugated small peptide vectors (SynB1) with doxorubicin and obtained highly promising results manifested by increased CNS uptake of doxorubicin. Another example can be a fusion of β-galactosidase protein and peptide, which led to the effective distribution to all tissues, including the brain [117].

Viral Vectors

There have been attempts to use adenoviruses, herpes simplex virus, or lentivirus as vectors for the delivery of cDNA to the target site, also in the brain. However, there are several problems such as immunological response, and inability to express the inserted gene for a long period, which should be addressed before effective application of viruses as drug carriers [70]. Another important limitation is the safety of viral vectors due to the death of patients in clinical trials [118]. Up to now, adeno-associated virus (AVV) vectors have been found to present exceptional safety in humans. In addition, they were effective in gene delivery in the brain. However, one should be aware that viruses normally cannot passively cross the BBB, though viruses can transfect the gene into the targeted cells [118]. Therefore, other ways of administration, such as injection in the CSF, have been developed. Research on new vectors is still ongoing, for example, a few AAV secrotypes showed the potential to bypass the BBB and target the cells of the CNS [119]. It is also worth noticing that viruses can act as inducers of opening the TJ via upregulation of chemokines as a precursor for infiltration of inflammatory cells into the CNS [120].

Molecular Trojan Horses

The "Trojan horse" approach is related to the modification of a specific drug that cannot freely pass the BBB but is attached to a special vector being capable of crossing the BBB. For instance, peptidomimetic monoclonal antibodies (mAbs) can utilize RMT across the BBB via endogenous peptide receptor transporters. By attaching a therapeutic molecule to the receptor-specific mAb, one can obtain improved transport across the BBB [113]. As presented by Pardridge et al. [121] the human insulin receptor (HIR) is one of the most potent molecular Trojan horses in the BBB. For instance, effective brain uptake was obtained in the rhesus monkey by attaching amurine 83–14 mAb to the HIR. Molecular Trojan horses can also be utilized for gene therapy. For instance, Zhang et al. used nonviral RNAi gene therapy directed against the human EGFR to treat brain cancer in a mouse model [122].

The complexes comprising a trojan horse and a nontransportable drug are also called chimeric peptides because the molecule is bifunctional. Chimeric peptide technique is based on the covalent coupling of a non-permeable drug to a BBB-transportable peptide vector (e.g., cationized albumin, insulin, transferrin, etc.) using a disulfide bond [123]. Such a chimeric peptide is then subjected to endocytosis by the ECs through receptor-mediated transcytosis and transported to the brain. In the brain tissue, disulfide reductases, cleave the active compounds from the peptide vector. Chimeric peptides can target various receptors in the BBB, e.g. insulin receptor, insulin-like growth factor, or transferrin [124].

Even better results in the CNS uptake can be obtained by combining nanocarriers with transportable vectors, such as TAT peptides. This combination enables to obtain a stable drug with improved CNS penetration and reduced side effects [70]. As an example, PEGylated ciprofloxacin showed improved brain uptake when its surface was modified with TAT peptide [125].

Physiological Approach

The physiological approach to brain drug delivery is based on the utilization of naturally occurring transport mechanisms across the BBB. These mechanisms include carrier-mediated transport (CMT), receptor-mediated transport, and adsorptive-mediated endocytosis (Figs. 3 and 4). By appropriately designing new molecules or modifying the existing ones so that they correspond to endogenous substrates and meet the structural requirements for the above-mentioned transporters, transport to the CNS can be effectively improved. In addition, the design of efflux pump inhibitors can prevent the removal of active substances from the CNS. Within this chapter, we will comprehensively review the current strategies using transporter and receptors present at the BBB (Fig. 4).

Delivery Via Endogenous Transporters

Endogenous transporters have been successfully utilized for the transport of molecules that can mimic endogenous substrates of several transporters localized in the BBB. As an example, levodopa, gabapentin, and melphalan, which structurally resemble phenylalanine, are effectively transported by LAT1 carrier [126]. In turn, the transport of basic drugs such as lidocaine or propranolol is mediated by organic cation transporter (OCT) [114]. Importantly, scientists have attempted to chemically modify drug molecules to resemble the structure of endogenous substrates. In this case, a CMT substrate can be utilized as a drug carrier, which provides the original structural characteristic of the substrate class for the targeted transporter. This research is a significant part of the interest of medicinal chemists [126] because targeting transporters with good transport capacity and low affinity has become an attractive strategy for the improvement of brain drug delivery [14]. To optimize drug delivery by CMT, the active compounds are designed as structural analogs of endogenous substrates for a particular transporter for efficient delivery to the brain [14]. For instance, the glutathione transporter has been used to transport glutathione-functionalized, PEGylated liposomes for the delivery of doxorubicin and paclitaxel [127]. Importantly, carrier-mediated transport of active molecules is limited to smaller drugs, which makes CMT harder to optimize for the delivery of large molecular weight drugs or macromolecules [128]. The other challenges associated with CMT are targeting the specific transporters without reducing affinity and meeting proper size criteria. Importantly, in the case of transporters with low transporter capacity, potential inhibition of drug uptake by high plasma levels of endogenous substrates should be considered [14]. In addition, some transporters in BBB are very selective in their stereochemical requirements for substrates and they cannot carry pseudo-substrates [114]. The basic characteristics of transporters localized in the BBB that could be utilized for brain-targeted drug delivery are presented in Table 1 and discussed in more detail below.

LAT1 Transporter

LAT1 transporter recognizes a carboxylic acid group and an amino group covalently linked to the same carbon atom, which is a part of an α-amino acid. LAT1 is selective for large neutral amino acids and requires a bulky hydrophobic side group. This group is very important as it allows the interaction with the cell membrane in such a way that the amino- and carboxylic-groups of the amino acids correctly align with the active site of the transporter. However,

Table 1 Basic characteristic of transport system of the BBB

Transporter	Encoding gene	Substrates	Remarks
GLUT1	SLC2A1	Glucose, 2-deoxyglucose, 3-*O*-methyl-glucose, galactose, and mannose	GLUT1 does not transport L-glucose; GLUT1 constitutes 90% of BBB Glucose transporters; expressed on luminal and abluminal sides of ECs
MCT1	SLC16A1	Lactate, pyruvate, ketone bodies, and monocarboxylic acids	Catalyzes the rapid transport across the plasma membrane of branched-chain oxo acids derived from leucine, valine and isoleucine, and the ketone bodies acetoacetate, beta-hydroxy-butyrate, and acetate. Located at luminal and abluminal side of ECs
LAT1	SLC7A5	Neutral L-amino acids (e.g., asparagine, glutamate, histidine, isoleucine, leucine, methionine, phenylalanine)	Preferential affinity for the large neutral amino acids; LAT1 is expressed at luminal and abluminal side of ECs,
CAT1	SLC7A1	Arginine, lysine, ornithine	High-affinity, low-capacity permease involved in the transport of the cationic amino acids. Located at the luminal side of ECs
CHT **CTL**	SLC5A7 SLC44A	Choline, quaternary ammonium molecules	CHT is a sodium ion- and chloride ion-dependent high-affinity transporter that mediates choline uptake for ACh synthesis in cholinergic neurons. CTL1-2 are expressed in brain microvascular ECs, and play a crucial role in cholinę transport in CNS
CNT2	SLC28A2	Adenosine, guanosine, uridine, inosine	Sodium-dependent and purine-selective transporter. Exhibits the transport characteristics of the nucleoside transport system cif or N1 subtype (N1/cif) (selective for purine nucleosides and uridine)
ENT	SLC29	Pyrimidine and purine nucleoside analogs	Nucleoside transporters are divided into two families: the Na+-dependent solute carrier family 28 (SLC28) and the equilibrative solute carrier family 29 (SLC29). SLC28 family transporters (CNT1, 2, and 3) display subtype-selective tissue expression patterns
NBT		Purine bases (adenine, guanine)	NBT transporter gene has not been cloned yet
OCT1-3	SLC22A1-3	Broad substrate profile, e.g. choline, acetylcholine, agmatine, monoamine neurotransmitters	OCTs are polyspecific, bi-directional, facilitative diffusional transporters. OCTs mediates Na+-independent transport of type I organic cations (protonated molecules). Although bi-directional, they typically behave as uptake transporters in vivo
OAT3	SLC22A8	Prostaglandins, uric acid, bile acids, conjugated hormones	Multispecific exchanger or antiporter that transports predominantly anionic substrates against a concentration gradient. Uptake of substrates depends on the outwardly directed concentration gradient of dicarboxylates (e.g. α-ketoglutarate)
OATP1A2 **OATP2B1**	SLCO1A2 SLCO2B1	OATPs transport a diverse range of organic anionic, neutral, cationic and amphipathic xenobiotic and endogenous molecules, including bile acids, conjugated sex steroids, T3 and T4, linear and cyclic peptides, mycotoxins, prostaglandin E2	Unidirectional organic anion uptake transporters

ACh acetylcholine, *ENT* equilibrative nucleoside transporters, *NBT* nucleobase transporter

glycine and alanine are not transported by LAT1 [129]. It has been found that the affinity (K_m) to LAT1 of L-enantiomers of phenylalanine and leucine was greater compared to the D-enantiomers. However, the transport rate (V_{max}) was similar to L- and D- enantiomers [130].

The most widely known substrate of LAT1 is the prodrug L-DOPA for the therapy of PD. Importantly, it is only 1% of an oral dose of L-DOPA, which is sufficient for therapeutic effect, while over 95% is decarboxylated to dopamine in the peripheral tissues [14]. In the CNS, the drug is decarboxylated by aromatic amino acid decarboxylase, and dopaminę is released in the brain [131]. Another example of a prodrug targeting LAT1 transporter is L-4-chlorokynurenine being a prodrug of 7-chlorokynurenic acid, which is an antagonist of NMDA. The prodrug is converted into chlorokynurenic acid by aminotransferase [132]. A similar strategy was applied in the case of tyrosine-based prodrug of nipecotic acid, which is a potent inhibitor of neuronal GABA uptake [133]. LAT1 transporter was also utilized to improve transport of ketoprofen, which was prepared in a form of a prodrug with L-tyrosine or L-lysine [134, 135].

Other well-known examples of LAT1 substrates include α-methyl DOPA, which is an antihypertensive drug, the chemotherapeutic melphalan, and antiepileptic gabapentin [114]. A lot of scientific work is also concentrated on the modification of commercially available drug molecules to increase transport to the CNS by the LAT1 transporter. Interestingly, the antimitotic drug, D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (D,L -NAM) has been found to have a much higher affinity for the transport system than the natural substrates and is preferentially transported into the brain [114]. Some other examples of drug modification into LAT1 substrate are shown in Fig. 8.

In summary, the chemical transformation of drug molecules into LAT1 substrates makes up a promising strategy for improving the delivery of drugs into the CNS. However, not all the above-mentioned examples of drug modifications were found to effectively cross the BBB. Most of the studies evaluated only the affinity towards LAT1 transporters using competitive inhibitors of LAT1 or large amounts of substrates. Importantly, in most of these studies, there is no information on the intra-brain distribution. Another important aspect to be considered is the lack of knowledge on the distribution of these modified drugs to other tissues.

GLUT 1 Transporter

It has been reported that GLUT1 makes up over 90% of BBB glucose transporters [113]. GLUT1 transporters have also been used in pre-clinical studies as transporters for chemically modified drug molecules. Due to the considerable expression and high capacity for CMT at the BBB, GLUT1 has become a promising target for prodrug delivery to the CNS. For instance, Bonina et al. [133] synthesized four new nipecotic acid esters, which were obtained by chemical conjugation with glucose and galactose (Fig. 9). In another study, Bonina et al. [136] prepared galactose and glucose esters of 7-chlorokynurenic acid, which is a potent glycine-N-methyl-D-aspartate (NMDA) receptor antagonist but shows weak activity after systemic administration. It was found that intraperitoneal administration of synthesized esters protected the tested animals from the seizures induced by NMDA, which means that they can be transported across the BBB. In turn, Halmos et al. [137] synthesized four glucose-chlorambucil derivatives, and studied their interactions with the GLUT1 transporter. All four compounds could reversibly inhibit [^{14}C]glucose uptake in a concentration-dependent manner, implying that they can interact with GLUT1 transporters, but not due to alkylation of a nucleophilic group of the hexose transporter [137]. Several *O*-methylsulphonyl derivatives of D-glucose were conjugated to busulphan to make it more hydrophilic; however, this strategy was not found to be effective [138]. Another example of drug modification to target GLUT1 transporter is the synthesis of glycosyl conjugates of dopamine and L-DOPA as potential anti-Parkinson pro-drugs (Fig. 9). Interestingly, the dopamine derivatives were found to be more effective in reversing reserpine-induced hypolocomotion in rats than L-DOPA or its conjugates [139].

Interesting results were also reported by Leveugle et al. [140] who have shown that degraded heparin, in contrast to full-length heparin, was able to cross the BBB, with tetrasaccharides and disaccharides being the most effective. This finding suggests that heparin-derived oligosaccharides can cross the BBB, depending on their molecular weight. Later on, it was reported that C3, an ultralow molecular weight heparin fragment, was detected in the brain and CSF 45 min after intravenous injection, which indicates that C3 can pass through the BBB [141]. It is also worth mentioning that non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen, and indomethacin, were conjugated with glucose at the 6-OH position for improved transport through the BBB with the aid of GLUT1 transporters [142].

Data on the use of glucose as a vector for peptide transport are also available in the literature. For instance, Kriss et al. [143] coupled *β*-D-glucose to the opioid peptide molecule possessing analgesic activity and found that these glycopeptides can penetrate the brain via the glucose moiety as a vector. In turn, Bilsky et al. attached simple sugars to enkephalins and found that these compounds possess improved penetration properties through the BBB [144].

Another fast-growing area of science is the functionalization of nano-enabled delivery systems with carbohydrate moieties to improve brain delivery of drugs [145]. As an example, liposomes modified with

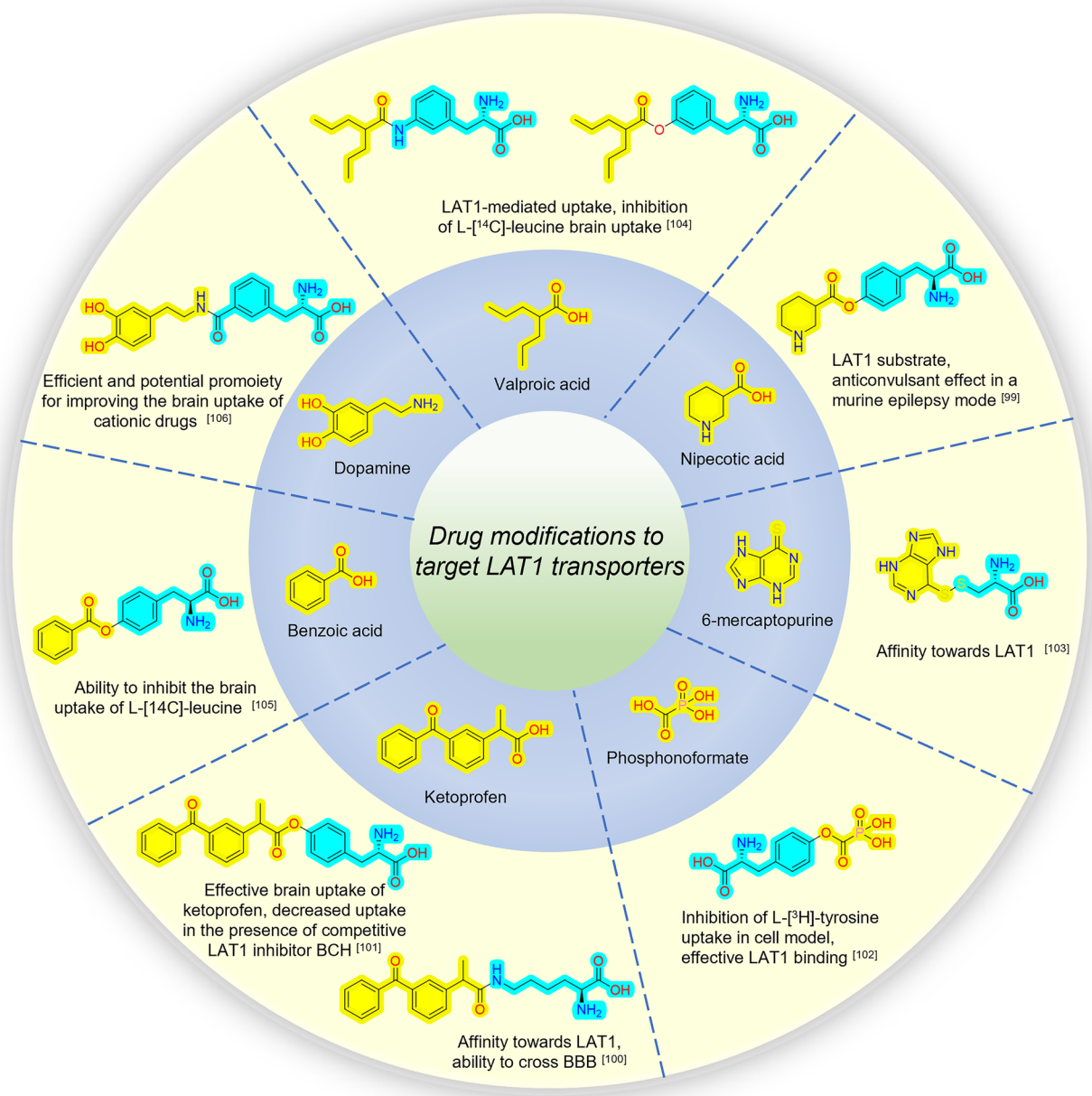

Fig. 8 Modifications of drug molecules to target LAT1 transporters

p-aminophenyl-α-D-mannopyranoside have been studied as carriers for the delivery of encapsulated drugs across the BBB via GLUT1 [146].

However, we should remember that designing prodrugs or derivatives having an affinity for the GLUT1 transporter is not straightforward. Those molecules should fill a few requirements, (i) hydrophilic drugs should be conjugated to the hydroxyl group at the C-6 position of D-glucose, (ii) the attached drug must be small and linked through a biodegradable bond, but ought to be stable enough to cross the BBB [14]. These limitations make the design of GLUT1 targeted prodrugs challenging and difficult.

MCT Transporters

The presence of MCT1 transporters in major organs such as the liver, brain, and intestine implies they may exert an influence on the pharmacokinetics of substrates, including pharmacologically active molecules. The presence of MCT1 transporters at the BBB suggests that they can

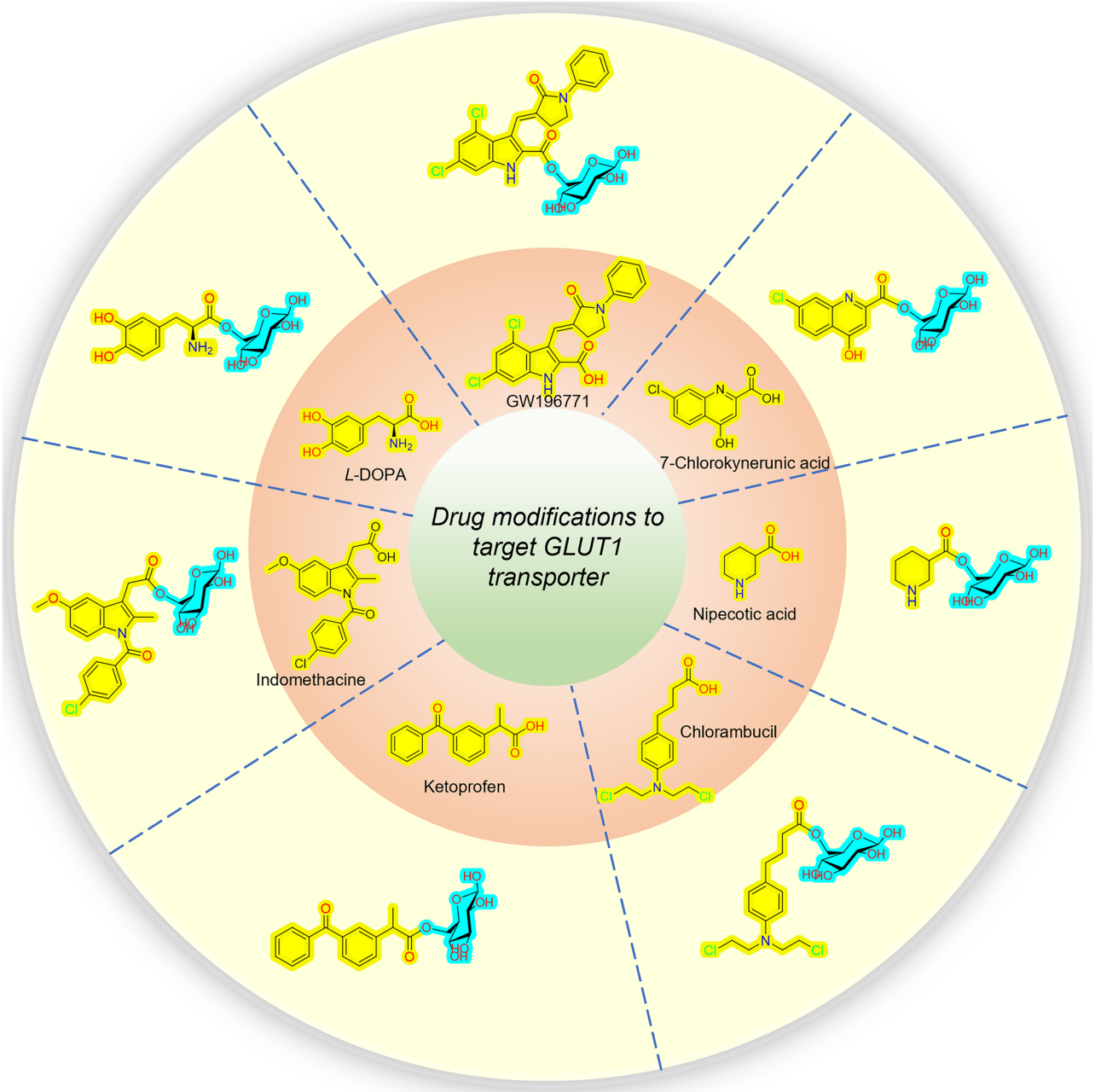

Fig. 9 Modifications of drug molecules to target GLUT1 transporter

constitute potential targets to improve the delivery of their substrates into the brain tissue [147]. Apart from the transport of endogenous short-chain monocarboxylates, MCT1 was also found to carry some drugs such as valproic acid, salicylate, bumetanide, nateglinide, simvastatin, and atorvastatin [148]. Kang et al. [149] have reported that acidic drugs such as valproic acid, benzoic acid, nicotinic acid, or beta-lactam antibiotics are transported into the brain utilizing MCT1 at the BBB in a pH-dependent manner. Later on, it was confirmed that the brain transport of acetic acid was profoundly inhibited by monocarboxylates, which indicates a role of MCTs in the transport of these compounds across the BBB [150]. Interestingly, the sleep disturbances after administration of simvastatin or lovastatin result from their ability to cross the BBB via MCTs. On the other hand, the ability of statins to permeate the brain can be used in the treatment of AD due to their antioxidant properties. It has been demonstrated that administration of atorvastatin significantly decreased lipoperoxidation, protein oxidation and nitration and also resulted in increased levels of glutathione

in parietal cortex of aged beagles [151]. Therefore, statins can be useful in the treatment of AD amelioration of oxidative damage.

MCTs may also act as the efflux transporters of certain drugs, e.g. probenecid, across the BBB. Deguchi et al. [152] reported that the restricted entry of probenecid into the brain is due to MCT-mediated efflux from the brain. It has also been found that 6-mercaptopurine is effluxed by MCTs in the BBB [153]. However, all the above-mentioned drugs have been serendipitously discovered to utilize MCT1 afterward and rational utilization of MCT1 in brain drug delivery remains to be seen in the future.

ENT Transporters

ENT transporters are highly important in the BBB because they are responsible for the transport of nucleosides and nucleobases necessary in the brain tissue. ENT1 and ENT2 are very well characterized and display broad substrate specificity for purine and pyrimidine nucleosides [27]. Chemically, ENT1 is selective for ribose or arabinose moieties, and sensitive to modifications at the C(2′) and C(5′) positions with the C(3′) hydroxyl being essential for substrate binding [154]. Structure–activity studies revealed that modifications at the C(3′)-position, a lack of conformational flexibility, and loss of the sugar ring are factors, which reduce the ability of compounds to function as transportable substrates [155]. ENT1 transporter participates also in the transport of several types of drugs, including antineoplastic drugs (e.g., cladribine, gemcitabine, fludarabine, and cytarabine), antiarrhythmic drugs (e.g., dilazep, dipyridamole), antiviral therapeutics (e.g., ribavirin, zalcitabine, zidovudine), and antihypertensive drugs (e.g., nifedipine) [154].

There are still several unresolved questions about ENT-mediated transport. For instance, it is not known whether the ENT transport is affected by regions of electronegativity, or it is sensitive to the orientation of the purine/pyrimidine ring about the glycosidic linkage. The knowledge on the role of hydrophobic interactions, or hydrogen bonding remains also unsolved [154]. Therefore, the understanding of ENT substrates has not been established yet.

CNT Transporters

Three isoforms of CNT transporters have been identified, CNT1, CNT2, and CNT3, which mediate Na^+-dependent co-transport of nucleosides and nucleoside analogs with CNT3 also mediating proton-driven co-transport. CNT1 mediates the uptake of pyrimidine nucleosides, CNT2 primarily transports purine nucleosides, and CNT3 transports both pyrimidine and purine nucleosides [156, 157]. Among them, CNT2 protein has been found at the luminal side of the BBB endothelium and the apical side of the choroid plexus epithelium [27]. Didanosine and ribavirin are examples of drugs being transported with the aid of CNT2 [158].

CNT3 mRNA has also been detected in the brain. However, its expression is not limited to this single organ. CNT3 takes part in the transport of nucleoside analogs that are used as chemotherapeutics or anti-HIV drugs, including cladribine, gemcitabine, zidovudine, ribavirin, mizoribine, clofarabine, fluoropyrimidine, formycin B, didanosine (ddI), maribavir, floxuridine, entecavir [159]. However, some studies show that antiviral drugs such as zidovudine are not so optimal substrates for nucleoside transporters, which was manifested by apparently lower affinity values than those reported for antiviral drugs [160]. The major difference between antineoplastic and antiviral drugs is that the latter lacks the 3'-hydroxyl group of the sugar, which is crucial for nucleoside recognition and translocation. This finding suggests that a slight structural transformation induces a dramatic change in transport efficiency [161, 162].

CAT1 Transporters

Cationic amino acid transporter is ubiquitous in the human body and transports arginine, lysine, valine, glutamate, and ornithine. Transport mostly basic amino acids, and, to a lower extent neutral and acidic amino acids [163]. CAT1 may function as a proton symporter [164].

Importantly, CAT1 was found to participate in cancer development; e.g., they promote cell growth, proliferation, and metastasis in colorectal and breast cancer [165]. Therefore, the scientists used CAT1 transporters as a way to interfere with tumor metabolism and exert anti-neoplastic activity [166]. As presented by Kozak, CAT1 may be a receptor for certain neurotrophic viruses [167]. Unlike the LAT 1 transporters [165], CAT1 has not been used to rationally design prodrugs for the targeted delivery of drugs to specific tissues.

OCT Transporters

Organic cation transporters (OCTs) are polyspecific facilitated diffusion transporters that mediate the cellular absorption and clearance of endogenous compounds and xenobiotics in humans [168]. All three isoforms of OCTs, OCT1-3 are expressed at the human brain microvascular endothelium; however, the exact localization of these transporters is not fully known [169]. Importantly, it has been found that OCTs may serve in the brain as a compensatory clearance system in case of monoamine spillover after high-affinity transporter blockade by antidepressants or psychostimulants. OCT2 and OCT3 were found to take part in a variety of central functions, including anxiety, response to stress, and antidepressant efficacy (e.g. response to imipramine, fluoxetine, or fluvoxamine)

[168]. In addition, these transporters are also engaged in other processes like osmoregulation and neurotoxicity. OCTs participate also in the transport of drugs. As an example, memantine, an N-methyl-d-aspartate (NMDA) receptor antagonist, is an established transport substrate and inhibitor of OCT1/OCT2 transporters and has been previously used in ischemic stroke [170]. Also, other drugs targeting NMDA receptors such as phencyclidine or ketamine may also function as OCT blockers [171]. OCT transporters may also be involved in the neurotoxicity induced by paraquat or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [172].

Because some drugs can concentrate several folds in the brain compared to blood, some assumptions have been made that they can interact with OCT transporters in the brain [173]. For instance, bupropion was found to suppress the activity of OCT2 in the brain. In turn, the activity of OCT3 was sensitive to clozapine and diazepam [173]. Other examples of drugs interacting with OCTs in the brain include antiviral drugs (aciclovir, lamivudine, abacavir) [174], antidiabetic drugs (metformin) [175], antineoplastic drug cisplatin [176], and antimalarial drug quinine [177].

OATP Transporters

The OATPs, a group of SLC transporters, are widely expressed throughout the body, also at the human BBB, with OATP1A2 and OATP2B1 expressed at the luminal side of ECs. OATPs mediate the uptake and efflux of endogenous molecules as well as xenobiotics, which possess amphipathic characteristics. The substrates for OATP transporters include prostaglandins, steroid and thyroid hormone conjugates, bile acids (e.g. bilirubin, cholic acid), and several therapeutic drugs [178]. As an example, OATPs transport 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors such as rosuvastatin, atorvastatin, or pitavastatin, which have been shown to exhibit both neuroprotective and antioxidant properties [179]. Other drug substrates of OATPs include antibiotics (e.g., erythromycin, tebipenem), β-blockers (acebutolol, atenolol, celiprolol), chemotherapeutics (e.g., methotrexate, imatinib), and anti-HIV drugs (e.g., lopinavir, saquinavir) [170]. OATP1A2, due to its expression in the BBB, may also be involved in the brain entry of opioids. Despite the moderate knowledge on the drug substrates for OATP, there is no information regarding transporter-specific inhibitors [180]. The above-mentioned findings imply that OATPs represent interesting candidates for drug uptake across the BBB; however, in many respects, the relevance of OATP in drug delivery across the BBB remains unknown [181].

OAT3 Transporters

Among ubiquitously expressed OAT transporters (primarily in the kidneys), OAT3 are present also at the abluminal side of the brain capillaries [182]. The major function of OATs is to transport organic anions against electrical and chemical forces. They allow for anions transport against their chemical gradient to obtain levels intracellularly several times higher than in the extracellular fluid [183]. OAT3 mediates the uptake of small molecule anions including xenobiotics, endogenous metabolites such as conjugates of signaling sex steroids, as well as vitamins and some plant-derived metabolites (e.g., flavonoids) or uremic toxins. Generally, the drug substrate spectrum for OAT3 is wide and includes NSAIDs, antibiotics (β-lactams, e.g., cefaclor, tazobactam), ciprofloxacin, zidovudine, methotrexate, and pravastatin. The current literature highlights that anion transporters may play an important role in the efficacy of multiple therapies as well as in modulating the intensity of adverse side effects associated with them [183]; however, most data is focused on the uptake, distribution or elimination, not the delivery to the CNS.

In the brain, OAT3 participates in the active efflux of several drugs, including PAH, benzylpenicillin, indoxyl sulfate, and homovanillic acid [182]. During the writing of this work, the authors did not find any literature examples of drug modifications to target OAT3 transporters in the brain.

Choline Transporters

Choline is transported by three different carriers including polyspecific OCT1-3 transporters with a low-affinity for choline, high-affinity choline transporter 1 (CHT), and choline transporter-like proteins (CTL1-5; SLC44A1-5). Brain ECs take up extracellular choline via intermediate-affinity CTL1 and low-affinity CTL2 transporters [184]. CHT1 is structurally related to the SGLT-sodium glucose cotransporter and participates in Na^+-dependent choline transport across the plasma membrane in neuronal tissues. This transport supplies choline for the synthesis of acetylcholine [185]. In turn, CTL1 has an intermediate affinity for choline, with a K_m in the low micromolar range. Similar to CHT1, CTL1 is inhibited by the choline analogue hemicholinium-3 (CH-3) [185]. The role of CTL1 in the CNS is still not fully understood, and some authors link this transporter with the development of neurodegenerative diseases.

Efflux Transporters

During the past few years, the role of efflux transporters, especially those present at the BBB or BCSFB, in the treatment of CNS diseases has been highly appreciated. Efflux transporters in the brain take part actively in the homeostasis

of endogenous compounds and protect the brain from potentially harmful xenobiotics. On the other hand, the activity of efflux transporters contributes to the decreased availability of administered drugs in the treatment of CNS diseases. Effective efflux of therapeutics from the brain by transporters is a frequent cause for the pharmaceutical industry to exclude novel compounds from the further development of CNS drugs. Additionally, elevated expression of efflux transporters that can be present in individual patients may cause therapeutic failure in CNS diseases [186]. Among the most widely known efflux transporters in the BBB are P-glycoprotein (Pgp, ABCB1), members of the MRP (ABCC) family, and breast cancer-related protein (BCRP/ABCG2). These transporters are associated with the limited brain penetrations of CNS-active drugs, which restricts drug effectiveness or may even result in mere drug resistance.

P-glycoprotein

P-gp, amphipathic cationic efflux pump, is localized in the luminal site of capillary EC of the BBB and serves as a general defense mechanism in the mammalian BBB, which reduces the penetration of harmful lipophilic compounds into the CNS. P-gp belongs to the ABC (ATP-binding cassette) superfamily comprising more than 30 families of transporters [187, 188]. P-gp is localized at the apical membrane of capillary ECs, which allows for immediate efflux of drugs back into the blood [189]. Importantly, the activity of P-gp at the apical membrane contributes to the reduced intracellular endothelial drug concentrations and, as a result, increases the concentration gradient between EC cytoplasm and brain extracellular space. Both these phenomena enhance the extrusion of drugs from the brain extracellular space, and the removal of the drugs into the blood [186].

Apart from the apical membrane of ECs, P-gp is also localized in the intracellular compartments. In cytoplasmic vesicles, P-gp may concentrate drugs in the interior of the vesicles and may thus sequester drugs away from their subcellular targets [190]. In the case of the cell membrane, high P-gp levels are expressed in caveolae [191].

During the past two decades, scientists have identified a large number of P-gp substrates. P-gp substrates are generally non-polar, weakly amphipathic compounds that vary considerably in molecular size [27]. Probably one of the most known examples of limiting the CNS penetration role of P-gp is loperamide, which is an opiate antidiarrheal agent. Loperamide is a substrate for P-gp and, therefore, cannot cross the BBB. High affinity for P-gp, and subsequent lack of CNS penetration make the drug to be available over the counter for a peripheral indication to reduce gut motility. Importantly, administration of even high doses of loperamide does not result in CNS effects, such as euphoria or dependance which is typical for other opiates [192]. P-gp is also essential in limiting the brain distribution of the nonsedating antihistaminic drug, fexofenadine [193]. In turn, the affinity towards P-gp makes domperidone, the dopaminergic antagonist, ineffective in the treatment of psychosis. Similarly, P-gp plays a major role in limiting the CNS penetration of HIV protease inhibitors and contributes to the reduced central activity of these drugs in the treatment of the neurological complications of HIV [193]. The other examples of P-gp substrates, including anticancer drugs, antidepressants, antiepileptic drugs, and immunosuppressants are presented in Table 2 [194–196]. Some studies have also suggested that P-pg participates in the transport of endogenous mediators such as steroids, bilirubin, or β-amyloid [197].

An important area related to P-gp is the development and use of its inhibitors to improve the efficacy of antineoplastic drugs. The presence of P-gp in tumors and at the BBB contributes to the multi-drug resistance (MDR) of brain tumors. Several anticancer drugs are P-gp substrates and, as a result, weakly pass the BBB, and do not exert anticancer effects. Using P-gp inhibitors in cancer therapy can therefore improve the pharmacokinetics of the anticancer drugs, and increase their CNS concentrations. Furthermore, the intracellular drug concentration in brain tumors can be elevated when the inhibitor also distributes to the brain tumor [187]. As an example, an increased in vitro BBB permeability was observed following simultaneous administration of the vinblastine and the P-gp inhibitor PSC833 [198]. Apart from PSC833, there are also other inhibitors such as verapamil, R-verapamil, cyclosporin-A, LY 335,979, GF 120,918, S 9788, and RU-486 [199]. Another possibility for interaction with P-gp is at its glycosylation sites [187].

Multidrug Resistance Proteins

The MRP transporters (MRP1-9) are encoded by the ABCC class of genes. Compared to P-gp and BCRP, the members of the MRP family are not ubiquitous in capillary ECs. The expression of MRP4 was detected at the luminal side of ECs in the brain. In an MRP4 knockout mouse model, topotecan concentration was elevated in the brain, which suggests the important role of MRP4 in topotecan distribution in the CNS [200]. Luminal expression of MRP5 in human BECs has also been confirmed [178]. MRPs mediate the transport of many endogenous substrates including hormones, prostaglandins, leukotrienes, and their conjugates (glucuronides, sulfates, and glutathione) [201]. Importantly, MRPs take part in the efflux transport of a diverse array of drugs [202], structurally being organic amphipathic anions. Cationic drugs can also be transported using MRP system, however, they are co-transported with glutathione (e.g., etoposide) [187]. The primary function of MRPs is to extrude xenobiotics from cells, thereby contributing to the development of the MDR phenotype [27]. Simultaneous administration of

Table 2 Basic characteristics of efflux transporters localized in BBB

Transporter	Localization in BBB	Encoding gene	Endogenous modulators	Substrates	Inhibitors
P-gp	Luminal membrane	MDR1/ABCB1 gene	VEGF, IL-6 downregulate P-gp expression; TNF-α upregulates P-gp	Erythromycin, valinomycin, cetirizine, fexofenadine, itraconazole, ketoconazole, risperidone, dexamethasone, hydrocortisone, ritonavir, nelfinavir, daunorubicin, paclitaxel, doxorubicin, vinblastine, atorvastatin, losartan, amiodarone, digoxin, verapamil, quinidine, cyclosporine A, tacrolimus	Cyclosporine, carvedilol, clarithromycin, chlorpromazine, amiodarone, dronedarone, itraconazole, ketoconazole, lapatinib, quinidine, reserpine, ritonavir, tacrolimus, tariquidar, elacridar, verapamil, valspodar (PSC833), zosuquidar (LY335979)
BCRP	Luminal membrane of brain capillary of endothelial cells	ABCG2 gene	17β-estradiol	Anthracyclines, daunorubicin, doxorubicin, irinotecan, topotecan, methotrexate, imatinib and other tyrosine kinase inhibitors, mitoxantrone, nucleoside analogs, prazosin, pantoprazole, statins, teriflunomide, chlorothiazide, ketamine, paliperidone, cladribine	Fumitremorgin C, Ko132, Ko134, Ko143, gefitinib, imatinib mesylate, novobiocin, estrone, 17ß-estradiol, ritonavir, omeprazole, ivermectin, cyclosporine, rilpivirine, darolutamide, curcumin, cyclosporine, elacridar
MRP1	Diversified expression—luminal and/or abluminal side of BBB, astrocytes, microglia. MRP1 is expressed on basolateral side of ECs. MRP2 and 3 are expressed on the luminal and basolateral side of ECs	MRP1/ABCC1 gene	GSH-conjugated catechol metabolites	Daunorubicin, doxorubicin, etoposide, methotrexate, teniposide, vincristine; drug- conjugates (glutathione, glucuronide, sulfate)	Cyclosporine A, probenecid, verapamil, levofloxacin, cimetidine
MRP2		MRP2/ABCC2 gene		Similar to MRP1 substrates, and azithromycin, cefodizime, ceftriaxone, valsartan, olmesartan, indinavir, lopinavir, ritonavir	Cyclosporine A, probenecid, verapamil, efavirenz
MRP3		MRP3/ABCC3 gene		Similar to MRP1 substrates, and fexofenadine, clopidogrel, resveratrol	Indomethacin, probenecid, sulfinpyrazone, efavirenz, furosemide, indomethacin
MRP4		MRP4/ ABCC4 gene		Methotrexate, 6-mercaptopurine, thioguanine, aciclovir, ritonavir, cefazolin, topotecan	Celecoxib, NSAIDs (e.g., Diclofenac), verapamil, telmisartan
MRP5		MRP5/ABCC5 gene		cAMP, cGMP, fluorescein, 6-mercaptopurine, thioguanine, rosuvastatin, atorvastatin	Probenecid, sildenafil, dipyridamole

VEGF vascular endothelial growth factor, *IL-6* interleukin 6, *TNF* tumor necrosis factor, *NSAIDs* non-steroidal anti-inflammatory drugs

MRP inhibitors like probenecid or MK-571 contributes to the enhanced drug penetration into the brain or inhibition of drug efflux from isolated brain ECs [203].

Breast Cancer Resistance Protein

The BCRP is an ABC transporter encoded by the ABCG2 gene and is located at the luminal site of capillary ECs [178]. Interestingly, BCRP is expressed at higher levels than P-gp in human capillary ECs [204]. However, its contribution to the transport of endogenous substrates or xenobiotics is not clearly understood as in the case of P-gp. BCRP is generally co-expressed with MDR1 and shares many of its substrates, inhibitors, and inducers. BCRP participates in the transport of some hormones and their conjugated metabolites [205] and urate. There is quite a large similarity between BCRP and P-gp in terms of drug substrates [178]. Substrates and inhibitors of BCRP include a wide range of clinically important and structurally diverse drugs, including rosuvastatin, glyburide, nitrofurantoin, dipyridamole, cimetidine, chlorothiazide, sulfasalazine, and leflunomide), or some dietary components [206]. One of the most studied functions of BCRP is being a mediator of drug resistance to chemotherapeutic agents. As an example, the role of BCRP in limiting brain penetration has been shown for prazosin and mitoxantrone [207]. Another substrate of BCRP is topotecan, an antineoplastic drug used to treat recurrent small-cell lung and ovarian cancer. High affinity towards BCRP in the BBB makes the drug weakly effective in the treatment of CNS cancers. Similar effects were also reported for imatinib [193].

BCRP inhibitors can be divided into three categories: (i) highly potent and relatively specific (e.g., fumitremorgin C and its analog Ko143), (ii) highly potent but relatively non-specific (e.g., GF120819), and (iii) more general inhibitors of ADME mechanisms (e.g., cyclosporine A and some of the anti-HIV protease inhibitors).

The exchange of metabolites and xenobiotics between blood and CSF occurs with the aid of transporters belonging to the ABC-transporters and SLC super-families. Similar to BBB, MRPs and P-gp are expressed in the choroid plexus [208]. MRPs are expressed on the apical or basolateral side of the epithelial cells and take part in the removal of metabolic waste products and harmful molecules out of the CNS. For instance, MRP1 protects the B-CSF barrier against heavy metal ions, toxins, and various xenobiotics [208]. In turn, P-gp is present on the apical side of CP epithelial cells and appears to have a different function than in BBB [209]. Importantly, SLC transporters participate in the neuroprotection of the B-CSF barrier. As reported by Ghersi-Egea et al. [210] plasma membrane monoamine transporter (PMAT; SLC29A4) removes endogenous monoamines such as serotonin, dopamine, and histamine from the CSF. In turn, organic anion transporter (OAT), and organic anion transporting polypeptide (OATP) take part in the removal of organic anions [5]. The issue of transporters in BCSFB has been well described by Solar et al. [208], who indicate that also other transporters, including MRP4 and MRP5, large neutral amino acid transporter LAT1 (SLC7a5), divalent metal transporter (DMT)-1, equilibrative nucleotide transporters (Ent)-1, peptide transporters (Pept)-2 are expressed in the CP epithelial cells.

In conclusion, efflux transporters at the BBB and the blood–CSF barrier limit the uptake of xenobiotics and participate in their extrusion. The localization at the apical membrane of capillary endothelial cells, efflux transporters play a major role in the removal of drug substrates back into the blood. For the newly developed compounds, it is highly important to establish whether a particular compound is a substrate or inhibitor of a certain transporter. Low affinity to BBB efflux transporters is beneficial for the development of CNS drugs that should reach therapeutic concentrations in the brain. In turn, high affinity to BBB efflux transporters is a desirable feature for the peripherally acting drugs to diminish CNS side effects. Antineoplastic drugs were among the first drugs that were discovered to be substrates of efflux transporters, including P-gp. The efflux of anticancer agents from the brain remains to be a major research challenge due to its potential to limit their therapeutic efficacy in the treatment of CNS cancer. Although the research methods are focused on identifying the criteria that allow a prediction of whether a particular compound is an efflux transporter substrate, reliable predictions are still not possible yet [186]. Another important area of research is the use of efflux transporter inhibitors to increase the concentration of selected drug molecules in the CNS. For instance, cyclosporin A is frequently used as a competitive P-gp inhibitor in experimental studies.

Taken all, influx and efflux transporter data together, CMT pathways have a crucial role in drug distribution and particularly in brain drug disposition [211]. Unfortunately, there is still a lack of knowledge (many orphan transporters) as well as a lack of appropriate tools to study these transporters, which is a major hurdle also in the development of successful brain-targeted pharmaceutics [212, 213]. Moreover, in the future, more attention should be paid to brain region-specific drug delivery and selective sub-brain transporter expression, since many diseases affect only certain parts of the brain. For example, the amygdala and hippocampus are affected by AD, and epilepsy, while Parkinson's disease (PD) is related to cerebellar and thalamic dysfunctions and MS with cortical problems. More importantly, disease-related or therapy-induced changes in the expression of influx or efflux transporters should be taken also into consideration, as they critically affect the brain pharmacokinetics of some CNS-active drugs [211, 214, 215]. Downregulation of influx

transporter may cause inadequate drug response, while upregulation of influxes and downregulation of effluxes may have toxic consequences. Furthermore, patient-specific differences, not only in genetics but also in physiology should be studied more carefully in the future, since transporters are regulated by circadian rhythms and hormone levels, which may have a significant effect on the best timing of the administration of particular medication to reach the brain within therapeutically relevant concentrations [216, 217].

Receptor-Mediated Delivery of Drugs

Capillary ECs in the brain have also proved to possess specific receptor-mediated transport mechanisms that potentially can be used as a way to carry therapeutic agents to the CNS (Fig. 3). The main receptor systems taking part in the RMT include TfR, IR, and LDLR. These receptors together with other newly discovered receptors can be exploited as targets for drug delivery to the brain. In order to take advantage of the endogenous RMT mechanism for drug delivery, the therapeutic agent must be linked with a molecule capable of targeting a particular receptor of the RMT system. This vector could be either the natural ligand or artificial ligands like antibodies or peptides [36]. For instance, antibodies that bind to the TfR can be transported into the brain and can function as carriers for the delivery of compounds, including proteins, genes, or drug-loaded nanoparticles [218]. In contrast to CMT, targeting RMT is not limited by the size of the conjugated therapeutic molecule because it employs vesicle-based transport [36].

Targeting the RMT system is an innovative and non-invasive approach for drug delivery that requires only an intravenous injection. RMT strategy has several advantages, for instance, the quantity of the drug being transported across the BBB can be increased substantially. In addition, a low dosage of drug can be used for effective transport, and adverse side effects can be minimized. Finally, RMT provides the versatility of targets (TfR, IR, folate receptor, lactoferrin receptor, etc.), and it can be exploited in conjugation with peptides, liposomes, polymer systems, or nanoparticles [219].

Transferrin Receptor

RMT using transferrin receptor (TfR) on the capillary ECs appears to be the best choice for improving the transport of biological therapeutics with high molecular weight to the brain. Since 1984, when Jefferies et al. [220] reported that ECs of the brain expressed TfRs as opposed to endothelial cells in other organs, the scientific interest in TfR has grown a lot. Selective expression of TfR in the BBB may contribute to the preferential accumulation of TfR-targeted substances in the brain [221]. Importantly, TfR may also improve the efficacy of transport of the already available antibody-based drugs used in the treatment of neurodegenerative diseases, e.g., via bi-specific antibody design [221].

The studies on targeting TfR can be divided into two categories regarding the type of drug delivery system, antibody-based drugs, and nanocarriers. The review of current and past literature shows that both endogenous ligand Tf, and a few antibodies or peptides can be used in TfR-targeted drug delivery. The primary advantage of using Tf is the fact that it binds to the physiological binding site on the Tf receptor and triggers the endogenous mechanism of endocytosis of the Tf-TfR complex into the brain ECs [221]. The previous studies on the mechanism of TfR-mediated transcytosis showed that neither the receptor nor the Tf itself transcytoses into the brain parenchyma from the luminal side of the vessel [222]. In turn, iron is released from its binding to Tf, removed out of the endosome, and subsequently out of the ECs [223]. Numerous studies have shown that the Tf molecule enables to improve drug uptake in the brain parenchyma using antibody constructs and nanocarriers. Therefore, there should be a particular mechanism allowing for the drug release from the TfR and its effective transport to the brain [221, 224]. However, nobody has reported such a mechanism. There are several examples of Tf-drug conjugates, including doxorubicin, chlorambucil, or mitomycin C (MMC) which were found to improve cytotoxicity of chemotherapeutics with a concomitant decrease in adverse effects [225].

Antibody OX26 is another often used TfR-targeting compound, which was originally developed by Jefferies and co-workers in the middle'80 s [226]. OX26 has been extensively tested as a TfR targeting molecule, both in a form of conjugates with various types of drugs or.

with different nanocarriers. These constructs have been used in preclinical studies on various diseases of CNS, including Parkinson's disease [227], Alzheimer's disease [228], or stroke [229]. Apart from Tf or OX26 antibody, small peptides have been tested to target TfR. These peptides did not interfere with the binding of endogenous Tf to TfR [230] but they were unstable in plasma. Importantly, they show a low affinity for TfR, which may make them less relevant compared to antibodies [36]. As an example, Israel et al. [231] compared the functionality of different peptides targeting the TfR and low-density lipoprotein receptor-related protein 1 (LRP1) and reported that the choice of a peptide is crucial for the subsequent uptake potential in the brain.

The exploitation of TfR as a target for improved drug delivery to the CNS has started thirty years ago. Friden et al. reported that conjugation of methotrexate with OX26 antibody resulted in elevated brain parenchymal levels of the drug compared to free drug [232]. These results were the first step in proving that TfR can be used for brain drug delivery. Later on, Friden et al. [233] published a paper

on improved brain delivery of nerve growth factor (NGF) conjugated to an anti-TfR antibody. The increased transport of NGF affected positively the survival of cholinergic and non-cholinergic neurons, which could be beneficial in the treatment of AD [233, 234]. This conjugate was also proved to be effective in the treatment of Huntington's disease [235]. Antibody OX26 was used for the TfR targeted delivery of numerous molecules, including brain-derived neurotrophic factor (BDNF) [236], epidermal growth factor (EGF) [237], fibroblast growth factor (FGF) [229], and vasoactive intestinal peptide (VIP) [238]. There are also other examples with the transport of antisense oligonucleotides [239], and peptide nucleic acids (PNAs) [240]. Despite these promising initial trials, there are a few doubts regarding the efficacy of this method. For instance, Moos and Morgan [241] reported that the constructs were localized in the ECs of the capillary wall with little evidence of transport into the brain parenchyma. This finding indicates that the OX26 antibody was bound too tightly to the TfR that release during the endosomal sorting process was unlikely. Similar reports on the lack of the OX26 presence in brain parenchyma were also published later [242, 243]. More recent studies suggest that this problem could be solved by modification of the affinity of the antibody towards the TfR because the antibody binding mode interferes with the transport process [102]. It ha also been suggested that the binding mode also could be changed through modulation of the avidity, engineering dual variable domain antibodies, or through introducing pH-sensitivity in the variable domain of the TfR-targeting antibody [36].

RMT through TfR has also been exploited using nanocarriers attached to the TfR-targeting molecules to improve the transport of cargo encapsulated in the nanocarrier across the BBB. The first studies included daunomycin-loaded liposomes, which were conjugated with OX26 antibody [244]. Various liposomal formulations have also been used as carriers of nucleic acids. It could be achieved by applying positively charged phospholipids, which allowed for incorporating both plasmid DNA or small RNAs [122, 245]. Besides liposomes, other nanoparticles may also be used to target TfR, including polymeric nanoparticles or gold nanoparticles. As an example, OX26 has been used to target various polymers such as poly-lactic acid (PLA)-PEG, PEG-polycaprolactone (PCL), and poly(lactic-co-glycolic acid) (PLGA) nanoparticles. This approach allowed for effective delivery of amphotericin B, Aß peptide, temozolomide across the BBB [246, 247]. Some other examples are presented in Table 3.

Folate Receptor

The folate receptor (FR) is a glycoprotein receptor that mediates the brain uptake of folic acid and other forms of folate through the process of endocytosis [219]. Folic acid is an endogenous ligand for FR, therefore it constitutes a promising and potent candidate for coating a drug delivery molecule. Such a construct could facilitate the transport of certain drugs through folate receptor-mediated endocytosis [248]. As an example, folic acid was conjugated to the nanocarrier containing doxorubicin and BCL-2 siRNA. This construct resulted in effective brain delivery of doxorubicin and BCL-2 siRNA [249]. In turn, PLGA nanoparticles modified with lactoferrin and folic acid allowed for improved delivery of etoposide across the BBB [250]. Also, other nanoscale drug delivery systems were found to improve brain delivery of chemotherapeutics, and as a consequence the efficacy of antineoplastic treatment in animal models.

Insulin Receptor

In the CNS, insulin is not only necessary to regulate glucose uptake and metabolism but also affects synaptogenesis and nerve growth and acts neuroprotectively. Insulin in the CNS is primarily derived from the blood, so it can cross the BBB. The mechanism of insulin transport has been studied thoroughly, and it occurs by receptor-mediated transcytosis via the signaling-related insulin receptor [38]. The insulin binding sites in IR on the luminal face of brain capillary ECs mediate the transport of insulin across the BBB and activate the signaling-related insulin receptor and insulin-like

Table 3 Receptor-mediated drug delivery using various nanocarriers

Nanocarrier	Targeting ligand	Targeting receptor	Drug used	Disease	Ref
PEG-PLGA NPs	Lactoferrin	LfR	-	PD	[274]
Liposome	RVG29 peptide	N-acetylcholine receptor	BPD	PD	[275]
Carbon dots	Tf	TfR	Doxorubicin	Astrocytoma	[276]
Self-assembly gold NPs	EGF peptide	EGFR	Doxorubicin	Brain tumor	[277]
Solid-lipid NPs	Monoclonal antibody	IR	Carmustine	Glioblastoma	[278]
Peptide-drug conjugate	Angiopep-2 peptide (An2)	LRP1 receptor	Morphine-6-glucuronide	Pain management	[279]

LfR lactoferrin receptor, *PD* Parkinson's disease, *BPD* N-3,4-bis (pivaloyloxy)-dopamine, *Tf* transferrin, *EGF* epidermal growth factor, *IR* insulin receptor, *LRP1* lipoprotein receptor

growth factor 1 (IGF-1) receptor. Since the endogenous metabolism of insulin is disrupted in situations where the receptor itself was targeted, therefore, monoclonal antibodies that have an affinity to an epitope of the IR should be used [219]. Similar to other transporters present at capillary cells of the BBB, conjugation of drugs to monoclonal antibodies that have affinity towards IR was found to be a promising strategy for improving drug delivery to the CNS. For instance, genetically modified murine monoclonal antibodies (83–14 MAb) linked to the human IR resulted in a chimeric HIRMAb, which could act as a carrier for neurotherapeutic drugs [251]. Another example are serum albumin nanoparticles conjugated with IR monoclonal antibodies and certain drug molecules. These conjugates were effectively transported across the BBB [219, 252]. Although there are a few examples of promising exploitation of IR as targets for drug delivery to the brain, researchers should be aware of its fundamental role in the maintenance of glucose homeostasis.

Lipoprotein Receptor

Low-density lipoprotein receptors (LDLR) are located on the surface of brain capillary ECs and act through the process of endocytosis. They transport not only LDL across the BBB but also take part in signaling pathways. They were also found to interact with several other molecules such as lactoferrin, melanotransferrin, TAT protein, or ApoE [253]. Similar to other receptors present on the BBB, LDLR can also be used as a target in drug delivery to the brain. Lipoprotein receptor–related proteins, particularly LRP1 and LRP2 can be used for drug delivery across the BBB [219].

Demeule et al. [254] reported the preparation of a construct consisting of hexa-peptide and polysorbate 80 nanoparticles (PBCA) and proved its effective transport through the BBB. It was presumed that the transport of this construct is based on the interaction with LDL receptors and subsequent endocytosis. Another example of targeting LDL receptors might be to use lactoferrin as a ligand. Lactoferrin is a multi-functional protein whose levels are higher in certain CNS diseases. PEG-PLA nanoparticles modified with lactoferrin showed therapeutic efficacy in the treatment of PD in rats [255]. Despite the promising above-mentioned examples, further research is needed to confirm the possibility of using LDLR in the targeted delivery of drugs to the CNS.

Leptin Receptor

Leptin is released into the blood from fat cells and enters the brain through the BBB via leptin receptors, which are expressed on the luminal face of ECs and function through endocytosis. In the CNS, leptin controls the energy balance, appetite, and thermogenesis [219]. Leptin receptors can also be used as a way to improve drug delivery to the brain. In this case, targeting ligands are peptides derived from leptin molecules [256]. Leptin analogs, like lep30 were conjugated with poly-L-lysine and PEG and further coupled to the nanoparticles, which resulted in improved transport efficacy through the BBB [257, 258]. In turn, lep70–89 conjugated with liposomes showed greater cellular uptake compared to unmodified liposomes and were found to bypass the degradation pathway of lysosomes [259].

Integrin Receptors

Integrin receptors are α-β-heterodimeric transmembrane receptors that take part in cell– ECM (extracellular matrix) and cell–cell adhesion by binding with ECM proteins (e.g. fibronectin (FN)) and transmembrane proteins on adjacent cells. As presented by Osada et al. β1-integrin-mediated adhesion of brain ECs to the ECM is critical for stabilizing claudin-5 in BBB tight junctions (TJs) and BBB integrity [9, 260]. Heterodimericity of integrin receptors is associated with variability in affinity for the respective ligands. It is possible for a single receptor to bind to more than one ligand, but it is also possible that several integrin receptors interact with the same ligand. Integrin receptors can be targeted using synthetic tri-peptide RGD, which consists of Arg-Gly-Asp [261]. This ligand has been conjugated to the nanodiamonds modified with fluorescent markers and was found to be effectively transported across the BBB facilitated by the integrin $\alpha_v\beta_3$ receptors present on glioblastoma cells [262]. RGD was also used in nanochain delivery systems for targeted delivery of doxorubicin [263].

Other Molecular Targets for RMT

RMT of drug molecules is not only limited to the above-mentioned receptors. The last decade has brought many examples of using other receptors localized on the ECs of BBB for targeted drug delivery. These include diphtheria-toxin receptor (DtR), nicotinic-acetylcholine receptors, scavenger receptors or interleukin receptors. For instance, CRM197, a non-toxic mutant form of diphtheria toxin, was reported to undergo transcytosis, and influence the PI3K/Akt signaling pathway affecting endocytosis [219]. PLGA nanoparticles loaded with CRM197 were shown to improve cellular accumulation. Tosi et al. reported that zidovudine was transported effectively across the BBB with the use of CRM197 that was coupled with PBCA nanoparticles [259]. Another example might be RVG29, a peptide targeting nicotinic-acetylcholine receptors, that was found to show higher brain transport when conjugated with nanoparticles [264]. One of the major drawbacks of the RMT system is its moderate transport capacity. Therefore, scientists are still working on new BBB RMT

targets that may have better BBB specificity. FC6, a single domain llama antibody (sdAb), can endocytose into human cerebrovascular ECs [265]. Subsequent in-depth studies reported that FC5 internalization was likely a receptor-mediated process occurring through a cell surface α(2,3)-sialoglycoprotein. These results suggest the potential of CF5 to cross the BBB [266].

In summary, the above examples of the use of receptors located on ECs cells in the BBB indicate the potential use of RMT in the targeted transport of drugs to the CNS. One significant drawback of the aforementioned RMT systems is their fairly ubiquitous expression contributing to peripheral organ uptake. In addition, the transport capacity of RMT is moderate, which leads to relatively low levels of brain uptake. We still find many inaccuracies and unresolved issues in RMT research. For instance, some authors report that TfR antibodies cross the BBB, while others state that the same antibodies remain confined in the capillary wall [232, 241]. Recent, more advanced studies using the latest antibody technology point out that the RMT route could be feasible [112, 267, 268]. Another important issue about RMT is the safety profile of these innovative constructs. It has been found that the administration of anti-TfR antibodies may induce severe side effects. For instance, anti-TfR antibodies were found to target not only TfRs on the surface of ECs but also on circulating reticulocytes, which contributed to reticulocyte destruction and hemolysis [230]. Some of the unfavorable safety results make it impossible to conduct clinical trials and force the researchers to look for new targets on the BBB for drug transport [269].

An important aspect to take into consideration when designing a drug delivery system like RMT is the fact of disrupted integrity of the BBB in the course of neurodegenerative diseases. Losing BBB integrity leads to leakage of administered probes in the areas of disease (e.g., neuroinflammation), which finally contributes to the diffusion of these molecules in the brain extracellular space [270]. As an example, liposomes conjugated with ApoE and β-amyloid peptides were effective in the treatment of animals suffering from induced multiple sclerosis, and experimental autoimmune encephalomyelitis (EAE) [271, 272]. Another vital issue about the disease-induced changes to the BBB integrity is the potential effect of this disease on the expression of receptors present on ECs [221].

In conclusion, although the RMT system has been studied for approximately 30 years, there is still much to be explained regarding the possible mechanism of transcytosis and reaching the brain parenchyma by delivered drugs. Some studies highlight the superiority of RMT as a drug delivery route over other strategies and some underscore its low relevance. However, one must agree that significant progress in this area is yet to come, and the results of these studies are expected with great anticipation.

Conclusions

The knowledge of the physiology and function of the BBB has broadened substantially in the past two decades. Actually, the BBB is regarded as not just a cellular wall, but a firmly organized and coordinated regulatory interface participating in numerous activities, including transport, secretion, and enzyme release.

As indicated in the literature review above, the effective transport across the BBB and subsequent reaching the brain parenchyma still remains difficult for scientists, as shown by the defined treatment strategies and unsatisfactory outcomes of subjects suffering from CNS disorders. Therefore, the future efforts of scientists should not be focused only on the development of novel formulations but also on getting to know the molecular details and determining the role, expression, and signaling of transporters localized in BBB. Using physiological pathways to deliver therapeutics via protein-facilitated routes or drug-antibody conjugates for RMT will allow crossing BBB of a wide range of pharmaceuticals. For instance, ANG1005 (known also as GRN1005) has been recognized as safe for patients with brain metastases or glioma in clinical phase I/II studies [273]. Another promising strategy for drug delivery to the CNS is nanocarrier systems. Because of their high drug loading capacity and the possibility of modifying their surface with different chemical groups, it is possible to use these nanostructures as delivery agents of hydrophilic pharmaceuticals, enzymes, peptides, or genetic material. Using the latest achievements of nanotechnology may help in the repurposing of many CNS-active compounds that could not meet the pharmacokinetic requirements for drug molecules.

In summary, given the tremendous progress that has been made over the past few years, we firmly believe that the combined efforts of multidisciplinary teams integrating medicinal chemists, molecular modelers, molecular biologists, and pharmaceutical scientists will ultimately lead to the development of effective strategies to deliver active molecules to the CNS.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Jorgensen C, Ulmschneider MB, Searson PC. Atomistic model of solute transport across the blood–brain barrier. ACS Omega. 2022;7:1100–12.
2. Upadhyay RK. Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int. Hindawi Publishing Corporation; 2014;2014.
3. Galea I. The blood – brain barrier in systemic infection and inflammation. Cell Mol Immunol. 2021;18:2489–501.
4. Brøchner CB, Holst CB, Møllgård K. Outer brain barriers in rat and human development. Front Neurosci. 2015;9:1–16.
5. Johanson C. Choroid plexus blood-CSF barrier: major player in brain disease modeling and neuromedicine. J Neurol Neuromedicine. 2018;3:39–58.
6. Tajes M, Ramos-Fernández E, Weng-Jiang X, Bosch-Morató M, Guivernau B, Eraso-Pichot A. The blood-brain barrier: structure, function and therapeutic approaches to cross it. Mol Membr Biol. 2014;31:152–67.
7. Cai P, Zheng Y, Sun Y, Zhang C, Zhang Q, Liu Q. New blood–brain barrier models using primary Parkinson's Disease rat brain endothelial cells and astrocytes for the development of central nervous system drug delivery systems. ACS Chem Neurosci. 2021;12:3829–37.
8. Förster C. Tight junctions and the modulation of barrier function in disease. Histochem Cell Biol. 2008;130:55–70.
9. Bony BA, Tarudji AW, Miller HA, Gowrikumar S, Roy S, Curtis ET, et al. Claudin-1-targeted nanoparticles for delivery to aging-induced alterations in the blood–brain barrier. ACS Nano. 2021;15:18520–31.
10. Petty Ma LE. Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation. Prog Neurobiol. 2002;68:311–23.
11. Haseloff RF, Blasig IE, Bauer HC, Bauer H. In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells in vitro. Cell Mol Neurobiol. 2005;25:25–39.
12. Barrier BB, Hajal C, Le RB, Kamm RD, Maoz BM. Biology and models of the blood-brain barrier. Annu Rev Biomed Eng. 2021;23:359–84.
13. Hawkins Bt DT. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev. 2005;57:173–85.
14. Mikitsh JL, Chacko AM. Pathways for small molecule delivery to the central nervous system across the blood-brain barrier. Perspect Medicin Chem [Internet]. 2014;11–24. Available from: https://doi.org/10.4137/PMC.S13384.
15. Brown LS, Foster CG, Courtney JM, King NE, Howells DW, Sutherland BA. Pericytes and neurovascular function in the healthy and diseased brain. Front Cell Neurosci. 2019;13:1–9.
16. Souza AD, Dave KM, Stetler RA, Manickam DS. Targeting the blood-brain barrier for the delivery of stroke therapies. Adv Drug Deliv Rev [Internet]. Elsevier B.V.; 2021;171:332–51. Available from: https://doi.org/10.1016/j.addr.2021.01.015.
17. Giaume C, Tabernero AMJ. Metabolic trafficking through astrocytic gap junctions. Glia. 1997;21:114–23.
18. Alahmari A. Blood-Brain barrier overview: structural and functional correlation. Neural Plast. 2021;6564585.
19. Luo L, Song S, Ezenwukwa CC, Jalali S, Sun B, Sun D. Ion channels and transporters in microglial function in physiology and brain diseases. Neurochem Int [Internet]. Elsevier Ltd; 2021;142:104925. Available from: https://doi.org/10.1016/j.neuint.2020.104925.
20. Tumani H, Hegen H. CSF albumin: albumin CSF/serum ratio (marker for blood-CSF barrier function). Cerebrospinal Fluid Clin Neurol. 2015;111–4.
21. Kadry H, Noorani B, Cucullo L. A blood – brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids Barriers CNS [Internet]. BioMed Central; 2020;17:1–24. Available from: https://doi.org/10.1186/s12987-020-00230-3.
22. Mccabe SM, Zhao N. The potential roles of blood–brain barrier and blood–cerebrospinal fluid barrier in maintaining brain manganese homeostasis. Nutrients. 2021;13:1833.
23. Saunders NR, Liddelow S DK. Barrier mechanisms in the developing brain. Front Pharmacol. 2012;3.
24. Pan W, Kastin AJ. Why study transport of peptides and proteins at the neurovascular interface. Brain Res. 2004;46:32–43.
25. Chen Y, Liu L. Modern methods for delivery of drugs across the blood-brain barrier. Adv Drug Deliv Rev. 2012;64:640–65.
26. Irudayanathan FJ, Wang N, Wang X, Nangia S. Architecture of the paracellular channels formed by claudins of the blood-brain barrier tight junctions. Ann N Y Acad Sci. 2017;1405:131–46.
27. Sanchez-Covarrubias L, Slosky L, Thompson B, Davis T, Ronaldson P. Transporters at CNS barrier sites: obstacles or opportunities for drug delivery?. Curr Pharm Des. 2014;20:1422–49.
28. Al Rihani SB, Darakjian LI, Deodhar M, Dow P, Turgeon J, Michaud V. Disease-induced modulation of drug transporters at the blood – brain barrier level. Int J Mol Sci. 2021;22:3742.
29. Aykac A. The function and expression of ATP-binding cassette transporters proteins in the Alzheimer's disease. Glob Med Genet. 2021;8:149–55.
30. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. Neurobiol Dis. 2010;37:13–25.
31. Peng W, Tan C, Mo L, Jiang J, Zhou W, Du J, et al. Glucose transporter 3 in neuronal glucose metabolism : health and diseases. Metab Clin Exp [Internet]. Elsevier Inc.; 2021;123:154869. Available from: https://doi.org/10.1016/j.metabol.2021.154869.
32. Huber J, Witt K, Hom S, Egleton R, Mark KDT. Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. Am J Physiol. 2001;280:1241–8.
33. Gosselet F, Loiola RA, Roig A, Rosell A, Culot M. Central nervous system delivery of molecules across the blood-brain barrier. Neurochem Int. 2021;144.
34. Charpentier JC, King PD. Mechanisms and functions of endocytosis in T cells. Cell Commun Signal [Internet]. BioMed Central; 2021;19:92. Available from: https://doi.org/10.1186/s12964-021-00766-3.
35. Candela P, Gosselet F, Miller F, Buee-Scherrer V, Torpier G, Cecchelli R, Fenart L. Physiological pathway for low-density lipoproteins across the blood-brain barrier: transcytosis through brain capillary endothelial cells in vitro. Endothelium. 2008;15:254–64.
36. Johnsen KB, Burkhart A, Melander F, Kempen PJ, Vejlebo JB, Siupka P, et al. Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma. Sci Rep [Internet]. Springer US; 2017;7:1–13. Available from: https://doi.org/10.1038/s41598-017-11220-1.
37. Pulgar VM. Transcytosis to cross the blood brain barrier, new advancements and challenges. Front Neurosci. 2019;13:1–9.
38. Rhea EM, Rask-Madsen C, Banks WA. Insulin transport across the blood–brain barrier can occur independently of the insulin receptor. J Physiol. 2018;596:4753–65.
39. Urayama A, Grubb JH, Sly WSBW. Developmentally regulated mannose 6-phosphate receptor-mediated transport of a lysosomal enzyme across the blood-brain barrier. Proc Natl Acad Sci USA. 2004;101:12658–63.
40. Molino Y, David M, Varini K, Jabes F, Gaudin N, Fortoul A. Use of LDL receptor-targeting peptide vectors for in vitro and in vivo cargo transport across the blood-brain barrier. FASEB J. 2017;31:1807–27.
41. Drappatz J, Brenner A, Wong ET, Eichler A, Schiff D, Groves MD. Phase I study of GRN1005 in recurrent malignant glioma. Clin Cancer Res. 2013;19:1567–76.
42. Golden PL, Maccagnan TJ, Pardridge WM. Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. J Clin Investig. 1997;99:14–8.

43. Pardridge WM. The blood-brain barrier: Bottleneck in brain drug development. NeuroRx. 2005;2:3–14.
44. Pinheiro RGR, Coutinho AJ, Pinheiro M. Nanoparticles for targeted brain drug delivery: what do we know?. Int J Mole. 2021;22:11654.
45. Jones EM, Polt R. CNS active O-linked glycopeptides. Front Chem. 2015;3:1–9.
46. René CA, Parks RJ. Delivery of therapeutic agents to the central nervous system and the promise of extracellular vesicles. Pharmaceutics. 2021;13.
47. Achar A, Myers R, Ghosh C. Drug delivery challenges in brain disorders across the blood – brain barrier : novel methods and future considerations for improved therapy. Biomedicines. 2021;9:1834.
48. Xiong B, Wang Y, Chen Y, Xing S, Liao Q, Chen Y, et al. Strategies for structural modification of small molecules to improve blood − brain barrier penetration : a recent perspective. J Med Chem. 2021;64(18):13152–73.
49. Huang L, Wells MC, Zhao Z. A practical perspective on the evaluation of small molecule CNS penetration in drug discovery. Drug Metab Lett. 2019;13:78–94.
50. Deo AK, Theil F, Nicolas J, UCBPS A, Foriest C, Alleud B-B. Confounding parameters in preclinical assessment of blood − brain barrier permeation: an overview with emphasis on species differences and effect of disease states. Mol Pharm. 2013.
51. Chen C, Zhou H, Guan C, Zhang H, Li Y, Jiang X, et al. Applicability of free drug hypothesis to drugs with good membrane permeability that are not efflux transporter substrates : a microdialysis study in rats. Pharmacol Res Perspect. 2020;8(2):e00575.
52. Heffron T. Challenges of developing small-molecule kinase inhibitors for brain tumors and the need for emphasis on free drug levels. Neuro Oncol. 2017;20:307–12.
53. Huang L, Li X, Roberts J, Janosky B. Differential role of P-glycoprotein and breast cancer resistance protein in drug distribution into brain, CSF and peripheral nerve tissues in rats differential role of P-glycoprotein and breast cancer resistance protein in drug distribution into brain. Xenobiotica. 2015;8254:547–55.
54. Morikawa A, Peereboom DM, Thorsheim HR, Samala R, Balyan R, Murphy CG, et al. Capecitabine and lapatinib uptake in surgically resected brain metastases from metastatic breast cancer patients: a prospective study. Neuro Oncol. 2015;17:289–95.
55. Van Bree J, Boer A, Danhof MBD. Drug transport across the blood-brain barrier. Pharm World Sci. 1993;15:2–9.
56. Misra A, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: a review. J Pharm Pharm Sci. 2003;6:252–73.
57. Levin VA. Relationship of octanol/water partition coefficient and molecular weight of rat brain capillary permeability. J Med Chem. 1980;23:682–4.
58. Pardridge WM. Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier: primary role of albumin bound hormone. Endocrinology. 1979;105:605–12.
59. Pardridge WM. Plasma protein-mediated transport of steroid and thyroid hormones. Am J Physiol. 1987;252:157–64.
60. Schaefer CP, Tome ME, Davis TP. The opioid epidemic: a central role for the blood brain barrier in opioid analgesia and abuse. Fluids Barriers CNS BioMed Central. 2017;14:1–11.
61. Madrid Y, Lander LF, Brem HLR. New directions in the delivery of drugs and other substances to the central nervous system. Adv Pharmacol. 1991;22:299–324.
62. Stanimirovic DB, Sandhu JK, Costain WJ. Emerging technologies for delivery of biotherapeutics and gene therapy across the blood-brain barrier. BioDrugs. 2018;32:547–59.
63. Payandeh J, Volgraf M. Ligand binding at the protein–lipid interface: strategic considerations for drug design. Nat Rev Drug Discov [Internet]. Springer US; 2021;20:710–22. Available from: https://doi.org/10.1038/s41573-021-00240-2.
64. Sakai H, Inoue H, Murata K, Toba T, Shimmyo Y, Narii N, Ueno SY, Igawa Y, Takemoto N. Fibroblast growth factor receptor modulators employing diamines with reduced phospholipidosis-inducing potential. Bioorg Med Chem. 2020;28:115562.
65. Fernandes J, Gattass CR. Topological polar surface area defines substrate transport by multidrug resistance associated protein 1 (MRP1 / ABCC1). J Med Chem. 2009;1:1214–8.
66. Rover S, Cesura AM, Huguenin P, Kettler R, Szente A. Synthesis and biochemical evaluation of N - (4-Phenylthiazol-2-yl) benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase. J Med Chem. 1997;2623:4378–85.
67. Tsang JE, Urner LM, Kim G, Chow K, Baufeld L, Faull K, et al. Development of a potent brain-penetrant EGFR tyrosine kinase inhibitor against malignant brain tumors. ACS Med Chem Lett. 2020;11:1799–809.
68. Gajbhiye KR, Pawar A, Mahadik KR, Gajbhiye V. PEGylated nanocarriers: a promising tool for targeted delivery to the brain. Colloids Surf B Biointerfaces. 2020;187.
69. Immordino ML, Cattel L. Stealth liposomes : review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine. 2006;1:297–315.
70. Kasinathan N, Jagani HV, Alex AT, Volety SM, Venkata RJ. Strategies for drug delivery to the central nervous system by systemic route. Drug Deliv. 2015;22:243–57.
71. Huwyler J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. Proc Natl Acad Sci U S A. 1996;93:14164–9.
72. Xing Y, Wen CY, Li ST, Xia ZX. Non-viral liposome-mediated transfer of brain-derived neurotrophic factor across the blood-brain barrier. Neural Regen Res. 2016;11:617–22.
73. McNeeley KM, Karathanasis E, Annapragada AV, Bellamkonda RV. Masking and triggered unmasking of targeting ligands on nanocarriers to improve drug delivery to brain tumors. Biomaterials. 2009;30:3986–95.
74. Xiang Y, Liang L, Wang X, Wang J, Zhang X, Zhang Q. Chloride channelmediated brain glioma targeting of chlorotoxin-modified doxorubicine-loaded liposomes. J Control Release. 2011;152:402–10.
75. Gao X, Tao W, Lu W, Zhang Q, Zhang Y, Jiang X, Fu S. Lectin-conjugated PEG–PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomaterials. 2006;27:3482–90.
76. Arduino I, Depalo N, Re F, Dal Magro R, Panniello A, Margiotta N, Fanizza E, Lopalco A, Laquintana V, Cutrignelli A, Lopedota AA, Franco M, Denora N. PEGylated solid lipid nanoparticles for brain delivery of lipophilic kiteplatin Pt(IV) prodrugs: an in vitro study. Int J Pharm. 2020;583:119351.
77. Lu W, Wan J, She Z, Jiang X. Brain delivery property and accelerated blood clearance of cationic albumin conjugated pegylated nanoparticle. J Control Release. 2007;118:38–53.
78. Clark AJ, Davis ME. Increased brain uptake of targeted nanoparticles by adding an acid-cleavable linkage between transferrin and the nanoparticle core. Proc Natl Acad Sci U S A. 2015;112:12486–91.
79. Zhang Y, Wang Y, Boado RJ, Pardridge WM. Lysosomal enzyme replacement of the brain with intravenous non-viral gene transfer. Pharm Res. 2008;25:400–6.
80. Li Y, He H, Jia X, Lu WL, Lou J, Wei Y. A dual-targeting nanocarrier based on poly(amidoamine) dendrimers conjugated with transferrin and tamoxifen for treating brain gliomas. Biomaterials. 2012;33:3899–908.
81. Huang R, Ke W, Liu Y, Jiang C, Pei Y. The use of lactoferrin as a ligand for targeting the polyamidoamine-based gene delivery system to the brain. Biomaterials. 2008;29:238–46.
82. Hattori Y. Delivery of plasmid DNA into tumors by intravenous injection of PEGylated cationic lipoplexes into tumor-bearing mice. Pharmacol & Pharm. 2016;07:272–82.

83. Coisne C, Hallier-Vanuxeem D, Boucau MC, Hachani J, Tilloy S, Bricout H, Monflier E, Wils D, Serpelloni M, Parissaux X, Fenart L, Gosselet F. Beta-cyclodextrins decrease cholesterol release and ABC-associated transporter expression in smooth muscle cells and aortic endothelial cells. Front Physiol. 2016;7:185.
84. Becker G, Da Silva S, Sabo A, Antal MC, Kemmel V, Monassier L. Blood–brain barrier permeability: is 5-hydroxytryptamine receptor type 4 a game changer?. Pharmaceutics. 2021.
85. Coisne C, Tilloy S, Monflier E, Wils D, Fenart L, Gosselet F. Cyclodextrins as emerging therapeutic tools in the treatment of cholesterol-associated vascular and neurodegenerative diseases. Molecules. 2016;21:1748.
86. Rautio J, Laine K, Gynther M, Savolainen J. Prodrug approaches for CNS delivery. AAPS J. 2008;10:92–102.
87. Zawilska JB, Wojcieszak J, Olejniczak AB. Prodrugs: a challenge for the drug development. Pharmacol Rep. 2013;65:1–14.
88. Dhokchawle BV, Gawad JB, Kamble MD, Tauro SJ, Bhandari AB. Promoieties used in prodrug design: a review. Indian J Pharm Educ Res. 2014;48:35–40.
89. Pavan B, Dalpiaz A, Ciliberti N, Biondi C, Manfredini S, Vertuani S. Progress in drug delivery to the central nervous system by the prodrug approach. Molecules. 2008;13:1035–65.
90. Lecomte JM, Costentin J, Vlaiculescu A, Chaillet P, Marcais-Collado H, Llorens-Cortes C, Leboyer M, Schwartz JC. Pharmacological properties of acetorphan, a parenterally active "enkephalinase" inhibitor. J Pharmacol Exp Ther. 1986;237(3):937–44.
91. Roques BP, Fournié-Zaluski MC, Soroca E, Lecomte JM, Malfroy B, Llorens C, Schwartz JC. The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. Nature. 1980;288(5788):286–8.
92. Yoshiharu D, Hideki H, Shinobu F, Takafumi N, Yoshinari Y, Shizuo Y, Ryohei K. Improved brain delivery of a nonsteroidal anti-inflammatory drug with a synthetic glyceride ester: a preliminary attempt at a CNS drug delivery system for the therapy of Alzheimer's disease. J Drug Target. 2000;8:371–81.
93. Singhal D, Morgan ME, Anderson BD. Role of brain tissue localized purine metabolizing enzymes in the central nervous system delivery of anti-HIV agents 2'-beta-fluoro-2',3'-dideoxyinosine and 2'-beta-fluoro-2',3'-dideoxyadenosine in rats. Pharm Res. 1997;14:786–92.
94. Shanmuganathan K, Koudriakova T, Nampalli S, Du J, Gallo JM, Schinazi RF, Chu CK. Enhanced brain delivery of an anti-HIV nucleosides 2'-F-ara-ddI by xanthine oxidase mediated biotransformation. J Med Chem. 1994;37:821–7.
95. Kao HD, Traboulsi A, Itoh S, Dittert L, Hussain A. Enhancement of the systemic and CNS specific delivery of L-dopa by the nasal administration of its water soluble prodrugs. Pharm Res. 2000;17:978–84.
96. Al-Ghananeem A, Traboulsi AA, Dittert LW, Hussain AA. Targeted brain delvery of 17β-estradiol via nasally administerd water soluble prodrugs. AAPS Pharm Sci Tech. 2002;3:1–8.
97. Bodor N. Redox drug delivery systems for targeting drugs to the brain. Ann New York Acad Sci. 1987;507:289–306.
98. Lu CT, Zhao YZ, Wong HL, Cai J, Peng L, Tian XQ. Current approaches to enhance CNS delivery of drugs across the brain barriers. Int J Nanomedicine. 2014;9:2241–57.
99. Prokai L, Prokai-Tatrai K, Bodor N. Targeting drugs to the brain by redox chemical delivery systems. Med Res Rev. 2000;20:367–416.
100. Dwibhashyam VSNA. Strategies for enhanced drug delivery to the central nervous system. Indian J Pharm Sci. 2008;70:145–53.
101. Bodor N, Prokai L, Wu WM, Farag H, Jonalagadda S, Kawamura M, Simpkins J. A strategy for delivering peptides into the central nervous system by sequential metabolism. Science. 1992;257:1698–700.
102. Hultqvist G, Syvänen S, Fang XT, Lannfelt L, Sehlin D. Bivalent brain shuttle increases antibody uptake by monovalent binding to the transferrin receptor. Theranostics. 2017;7:308–18.
103. Faresjö R, Bonvicini G, Fang XT, Aguilar X, Sehlin D, Syvänen S. Brain pharmacokinetics of two BBB penetrating bispecific antibodies of different size. Fluids Barriers CNS [Internet]. BioMed Central; 2021;18:26. Available from: https://doi.org/10.1186/s12987-021-00257-0.
104. Kariolis MS, Wells RC, Getz JA, Kwan W, Mahon CS, Tong R, Kim DJ, Srivastava A. Brain delivery of therapeutic proteins using an Fc fragment blood–brain barrier transport vehicle in mice and monkeys. Sci Transl Med. 2020;12:1359.
105. Kouhi A, Pachipulusu V, Kapenstein T, Hu P, Epstein AL, Khawli LA. Brain disposition of antibody-based therapeutics: dogma, approaches and perspectives. Int J Mol Sci. 2021;22:1–24.
106. Simonneau C, Duschmalé M, Gavrilov A, Brandenberg N, Hoehnel S, Ceroni C, et al. Investigating receptor-mediated antibody transcytosis using blood–brain barrier organoid arrays. Fluids Barriers CNS. 2021;18:1–17.
107. Syvänen S, Hultqvist G, Gustavsson T, Gumucio A, Laudon H, Söderberg L, et al. Efficient clearance of A β protofibrils in A β PP-transgenic mice treated with a brain-penetrating bifunctional antibody. Alzheimer's Res Ther. 2018;10:49.
108. Gustavsson T, Syvänen S, Callaghan PO. SPECT imaging of distribution and retention of a brain-penetrating bispecific amyloid-β antibody in a mouse model of Alzheimer's disease. Transl Neurodegener. 2020;9:37.
109. Pizzo ME, Wolak DJ, Kumar NN, Brunette E, Brunnquell CL, Hannocks M, et al. Intrathecal antibody distribution in the rat brain : surface diffusion, perivascular transport and osmotic enhancement of delivery. J Physiol. 2018;3:445–75.
110. Ding S, Khan AI, Cai X, Song Y, Lyu Z, Du D, et al. Overcoming blood – brain barrier transport : Advances in nanoparticle-based drug delivery strategies. Mater Today [Internet]. Elsevier B.V.; 2020;37:112–25. Available from: https://doi.org/10.1016/j.mattod.2020.02.001.
111. Cavaco M, Gaspar D, Arb Castanho M, Neves V. Antibodies for the treatment of brain metastases, a dream or a reality?. Pharmaceutics. 2020;12:62.
112. Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, et al. Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. Neuron. 2014;81:49–60.
113. Patel MM, Goyal BR, Bhadada SV, Bhatt JS, Amin AF. Getting into the brain: approaches to enhance brain drug delivery. CNS Drugs. 2009;23:35–58.
114. Begley DJ. Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. Pharmacol Ther. 2004;104:29–45.
115. Torchilin VP, Rammohan R, Weissig V, Leuchenko TSTAT. peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. Proc Natl Acad Sci. 2000;98:8786–91.
116. Rousselle C, Clair P, Lefauconnier J. New advances in the transport of doxorubicin through the blood-brain barrier by a peptide vector-mediated strategy. Mol Pharmacol. 2000;57:679–86.
117. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. In vivo protein transduction: delivery of a biologically active protein into the mouse. Science. 1999;285:1569–72.
118. Dong X. Current strategies for brain drug delivery. Theranostics. 2018;8:1481–93.
119. Vagner T, Dvorzhak A, Wójtowicz A. Systemic application of AAV vectors targeting GFAP-expressing astrocytes in Z-Q175-KI Huntington's disease mice. Mol Cell Neurosci. 2016;77:76–86.
120. Kuang Y, Lackay SN, Zhao L, Fu ZF. Role of chemokines in the enhancement of BBB permeability and inflammatory infiltration after rabies virus infection. Virus Res. 2009;144:18–26.

121. Pardridge WM, Kang YS, Buciak J. Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries in vitro and rapid transcytosis through the blood-brain barrier in vivo in the primate. Pharm Res. 1995;12:807–16.
122. Zhang Y, Zhang Y, Bryant J, Charles A, Boado RJ, Pardridge W. Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. Clin Cancer Res. 2004;10:3667–77.
123. Bellettato CM, Scarpa M. Possible strategies to cross the blood–brain barrier. Ital J Pediatr. 2018;44.
124. Pardridge WM. Brain drug targeting and gene technologies. Jpn J Pharmacol. 2001;87:97–103.
125. Liu L, Guo KLJ. Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier. Biomaterials. 2008;29:1509–17.
126. He Q, Liu J, Liang J, Liu X, Li W, Liu Z, et al. Towards improvements for penetrating the blood–brain barrier—recent progress from a material and pharmaceutical perspective. Cells. 2018;7:24.
127. Agrawal M, Ajazuddin, Tripathi DK, Saraf S, Saraf S, Antimisiaris SG, Mourtas S, Hammarlund-Udenaes M, Alexander A. Recent advancements in liposomes targeting strategies to cross blood-brain barrier (BBB) for the treatment of Alzheimer's disease. J Control Release. 2017;260:61–77.
128. Angela R. Jones EVS. Blood-brain barrier transport of therapeutics via receptor-mediation. Pharm Res [Internet]. 2007;24:1759–71. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf. Accessed 20 Nov 2021.
129. Smith QR, Stoll J. Molecular characterization of amino acid transporters at the blood-brain barrier. Brain Barrier Syst. 1999;45:303–20.
130. Puris E, Gynther M, Auriola S, Huttunen KM. L-Type amino acid transporter 1 as a target for drug delivery. Pharm Res. 2020;37.
131. Djaletti R, Melamed E. New therapies for Parkinson's disease. J Neurol. 2001;248:357–62.
132. Hokari M, Wu HQ, Schwarcz R, Smith QR. Facilitated brain uptake of 4-chlorokynureine and conversion to 7-chlorokynurenic acid. Neuropharmacol Neurotoxicol. 1996;8:15–8.
133. Bonina FP, Arenare L, Palagiano F, Salia A, Nava F, Trombetta D, de Caprariis P. Synthesis, stability and pharmacological evaluation of nipecotic acid prodrugs. J Pharm Sci. 1999;88:561–7.
134. Gynther M, Jalkanen ALM. Brain uptake of ketoprofen-lysine prodrug in rats. Int J Pharm. 2010;399:121–8.
135. Gynther M, Laine K, Ropponen J, Leppänen J, Mannila A, Nevalainen T, Savolainen J, Järvinen T, Rautio J. Large neutral amino acid transporter enables brain drug delivery via prodrugs. J Med Chem. 2008;51:932–6.
136. Bonina FP, Arenare L, Ippolito R, Boatto G, Battaglia G, Bruno V, de Caprariis P. Synthesis, pharmacokinetics and anticonvulsant activity of 7-chlorokynurenic acid prodrugs. Int J Pharm. 2000;202:79–88.
137. Halmos T, Santarromana M, Antonakis K, Scherman D. Synthesis of glucose chlorambucil derivatives and their recognition by the human GLUT1 glucose transporter. Eur J Pharm. 1996;318:477–84.
138. Halmos T, Santarromana M, Antonakis K, Scherman D. Synthesis of O-methylsulfonyl derivatives of D-glucose as potential alkylating agents for targeted drug delivery to the brain. Evaluation of their interaction with the human erythrocyte GLUT1 hexose transporter. Carbohydr Res. 1997;299:15–21.
139. Bonina F, Puglia C, Rimoli MG, Melisi D, Boatto GNM, Malignano ALRG. Glycosyl derivatives of dopamine and L-dopa as antiparkinson prodrugs: synthesis, pharmacological activity and in vitro stability studies. J Drug Target. 2003;11:25–36.
140. Leveugle B, Ding W, Laurence F, Dehouck MP, Scanameo A, Cecchelli R, Fillit H. Heparin oligosaccharides that pass the blood-brain barrier inhibit beta-amyloid precursor protein secretion and heparin binding to beta-amyloid peptide. J Neurochem. 1998;70:736–44.
141. Ma Q, Dudas B, Hejna M, Cornelli U, Lee JM, Lorens S, Mervis R, Hanin I, Fareed J. The blood-brain barrier accessibility of a heparin-derived oligosaccharides C3. Thromb Res. 2002;105:447–53.
142. Gynther M, Ropponen J, Laine K, Leppänen J, Haapakoski P, Peura L, Järvinen T, Rautio J. Glucose promoiety enables glucose transporter mediated brain uptake of ketoprofen and indomethacin prodrugs in rats. J Med Chem. 2009;52:3348–53.
143. Kriss CT, Lou B-S, Szabo LZ, Mitchell SA, Hruby VJ, Polt R. Enkephalinbased drug design: Conformational analysis of O-linked glycopeptides by NMR and molecular modeling. Tetrahedron Assymetr. 2000;11:9–25.
144. Bilsky E, Egleton RD, Mitchell SA, Palian MM, Davis P, Huber JD, Jones H, Yamamura HI, Janders J, Davis TP, Porreca F, Hruby VJ, Polt R. Enkephalin glycopeptide analogues produce analgesia with reduced dependence liability. J Med Chem. 2000;47:2586–90.
145. Patching SG. Glucose transporters at the blood-brain barrier: function, regulation and gateways for drug delivery. Mol Neurobiol. 2017;54:1046–77.
146. Hao ZF, Cui YX, Li MH, Du D, Liu MF, Tao HQ, Li S, Cao F. Liposomes modified with P-aminophenyl-α-D-mannopyranoside: a carrier for targeting cerebral functional regions in mice. Eur J Pharm Biopharm. 2013;84:505–16.
147. Vijay N, Morris ME. Role of monocarboxylate transporters in drug delivery to the brain. Curr Pharm Des [Internet]. 2014;20:1487–98. Available from: https://doi.org/10.1016/j.earlhumdev.2015.09.003, https://doi.org/10.1016/j.earlhumdev.2014.01.002, https://doi.org/10.1016/S0378-3782(12)70006-3, http://www.sciencedirect.com/science/article/pii/S2341287914000763.
148. Morris ME, Felmlee MA. Overview of the proton-coupled MCT (SLC16A) family of transporters: characterization, function and role in the transport of the drug of abuse γ-Hydroxybutyric acid. AAPS J. 2008;10:311–21.
149. Kang YS, Terasaki T, Tsuji A. Acidic drug transport in vivo through the blood-brain barrier. A role of the transport carrier for monocarboxylic acids. J Pharmacobiodyn. 1990;13:158–63.
150. Terasaki T, Takakuwa S, Moritani S, Tsuji A. Transport of monocarboxylic acids at the blood brain barrier: studies with monolayers of primary cultured bovine brain capillary endothelial cells. J Pharmacol Exp Ther. 1991;258:932–7.
151. Barone E, Cenini G, Di Domenico F. Long-term high-dose atorvastatin decreases brain oxidative and nitrosative stress in a preclinical model of Alzheimer disease: a novel mechanism of action. Pharmacol Res. 2011;63:172–80.
152. Deguchi Y, Nozawa K, Yamada S, Yokoyama Y, Kimura R. Quantitative evaluation of brain distribution and blood-brain barrier efflux transport of probenecid in rats by microdialysis: possible involvement of the monocarboxylic acid transport system. J Pharmacol Exp Ther. 1997;280:551–60.
153. Deguchi Y, Yokoyama Y, Sakamoto T. Brain distribution of 6-mercaptopurine is regulated by the efflux transport system in the blood-brain barrier. Life Sci. 2000;66:649–62.
154. Boswell-Casteel RC, Hays FA. Equilibrative nucleoside transporters – a review. Nucleosides Nucleotides Nucleic Acids. 2017;36:7–30.
155. Gati WP, Misra HK, Knaus EE, Wiebe LI. Structural modifications at the 2'- and 3'- positions of some pyrimidine nucleosides as determinants of their interaction with the mouse erythrocyte nucleoside transporter. Biochem Pharmacol. 1984;33:3325–31.

156. Hermann R, Krajcsi P, Fluck M, Seithel-Keuth A, Bytyqi A, Galazka A, et al. Review of transporter substrate, inhibitor, and inducer characteristics of cladribine. Clin Pharmacokinet [Internet]. Springer International Publishing; 2021; Available from: https://doi.org/10.1007/s40262-021-01065-3.
157. Owen RP, Badagnani I, Giacomini KM. Molecular determinants of specificity for synthetic nucleoside analogs in the concentrative nucleoside transporter, CNT2. J Biol Chem [Internet]. Â© 2006 ASBMB. Currently published by Elsevier Inc; originally published by American Society for Biochemistry and Molecular Biology.; 2006;281:26675–82. Available from: https://doi.org/10.1074/jbc.M513421200.
158. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: The organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br J Pharmacol. 2012;165:1260–87.
159. Ritzel MWL, Ng AML, Yao SYM, Graham K, Loewen SK, Smith KM, et al. Molecular identification and characterization of novel human and mouse concentrative Na+-nucleoside cotransporter proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (System cib). J Biol Chem. 2001;276:2914–27.
160. Molina-Arcas M, Casado F, Pastor-Anglada M. Nucleoside transporter proteins. Curr Vasc Pharmacol. 2009;7:426–34.
161. Cano-Soldado P, Larráyoz IM, Molina-Arcas M, Casado FJ, Martinez-Picado J, Lostao MP, et al. Interaction of nucleoside inhibitors of HIV-1 reverse transcriptase with the concentrative nucleoside transporter-1 (SLC28A1). Antivir Ther. 2004;9:993–1002.
162. Chang C, Swaan PW, Ngo LY, Lum PY, Patil SD, Unadkat J. Molecular requirements of the human nucleoside transporters hCNT1, hCNT2, and hENT1. Mol Pharmacol. 2004;65:558–70.
163. Huang Y, Kang BN, Tian J, Liu Y, Luo HR, Hester L, et al. The cationic amino acid transporters CAT1 and CAT3 mediate NMDA receptor activation-dependent changes in elaboration of neuronal processes via the mammalian target of rapamycin mTOR pathway. J Neurosci. 2007;27:449–58.
164. Zaragozá R. Transport of amino acids across the blood-brain barrier. Front Physiol. 2020;11:1–11.
165. Zhang L, Sui C, Yang W, Luo Q. Amino acid transporters: emerging roles in drug delivery for tumor-targeting therapy. Asian J Pharm Sci. 2020;15:192–206.
166. Werner A, Pieh D, Echchannaoui H, Rupp J, Rajalingam K, Theobald M, et al. Cationic amino acid transporter-1-mediated arginine uptake is essential for chronic lymphocytic leukemia cell proliferation and viability. Front Oncol. 2019;9:1–14.
167. Kozak CA. Naturally occurring polymorphisms of the mouse gammaretrovirus receptors CAT-1 and XPR1 alter virus tropism and pathogenicity. Adv Virol. 2011;2011:14–7.
168. Couroussé T, Gautron S. Role of organic cation transporters (OCTs) in the brain. Pharmacol Ther. 2015:94–103.
169. Koepsell H. Polyspecific organic cation transporters: their functions and interactions with drugs. Trends Pharmacol Sci. 2004;25:375–81.
170. Williams EI, Betterton RD, Davis TP, Ronaldson PT. Transporter-mediated delivery of small molecule drugs to the brain: a critical mechanism that can advance therapeutic development for ischemic stroke. Pharmaceutics. 2020;12.
171. Amphoux A, Vialou V, Drescher E, Brüss M, La Cour CM, Rochat C, et al. Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. Neuropharmacology. 2006;50:941–52.
172. Cui M, Aras R, Christian WV, Rappold PM, Hatwar M, Panza J, et al. The organic cation transporter-3 is a pivotal modulator of neurodegeneration in the nigrostriatal dopaminergic pathway. Proc Natl Acad Sci U S A. 2009;106:8043–8.
173. Haenisch B, Drescher E, Thiemer L, Xin H, Giros B, Gautron S, et al. Interaction of antidepressant and antipsychotic drugs with the human organic cation transporters hOCT1, hOCT2 and hOCT3. Naunyn Schmiedebergs Arch Pharmacol. 2012;385:1017–23.
174. Minuesa G, Volk C, Molina-Arcas M, Gorboulev V, Erkizia I, Arndt P, et al. Correction to "transport of lamivudine [(-)-β-L-2′, 3′-dideoxy-3′-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3. J Pharmacol Exp Ther. 2009;329:1187.
175. Kimura N, Masuda S, Tanihara Y, Ueo H, Okuda M, Katsura T, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. Drug Metab Pharmacokinet. 2005;20:379–86.
176. Ciarimboli G, Deuster D, Knief A, Sperling M, Holtkamp M, Edemir B, et al. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. Am J Pathol. 2010;176:1169–80.
177. Wenge B, Geyer J, Bönisch H. Oxybutynin and trospium are substrates of the human organic cation transporters. Naunyn Schmiedebergs Arch Pharmacol. 2011;383:203–8.
178. Torres-Vergara P, Escudero C, Penny J. Drug transport at the brain and endothelial dysfunction in preeclampsia: Implications and perspectives. Front Physiol. 2018;9:1–12.
179. Thompson BJ, Sanchez-Covarrubias L, Slosky LM, Zhang Y, Laracuente M, Ronaldson PT. Hypoxia/reoxygenation stress signals an increase in organic anion transporting polypeptide 1a4 (Oatp1a4) at the blood-brain barrier: Relevance to CNS drug delivery. J Cereb Blood Flow Metab. 2014;34:699–707.
180. Schäfer AM, Meyer HE, Grube M. Expression and Function of Organic Anion Transporting Polypeptides in the Human Brain: Physiological and Pharmacological Implications. Pharmaceutics. 2021;13:384.
181. Kinzi J, Grube M, Zu Schwabedissen HEM. OATP2B1 – The underrated member of the organic anion transporting polypeptide family of drug transporters?. Biochem Pharmacol [Internet]. Elsevier Inc.; 2021;188:114534. Available from: https://doi.org/10.1016/j.bcp.2021.114534.
182. Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: Role of the solute carrier family. NeuroRx. 2005;2:73–85.
183. Mor AL, Kaminski TW, Karbowska M, Pawlak D. New insight into organic anion transporters from the perspective of potentially important interactions and drugs toxicity. J Physiol Pharmacol. 2018;69:307–24.
184. Inazu M. Functional expression of choline transporters in the blood-brain barrier. Nutrients. 2019;11.
185. Hedtke V, Bakovic M. Choline transport for phospholipid synthesis: an emerging role of choline transporter-like protein 1. Exp Biol Med [Internet]. 2019;244:655–62. Available from: https://doi.org/10.1177/1535370219830997.
186. Abel Lajtha MR. Handbook of Neurochemistry and Molecular Neurobiology. Pap. Knowl. Towar. a Media Hist. Doc: Springer; 2007.
187. De Boer AG, Van der Sandt ICJ, Gaillard PJ. The role of drug transporters at the blood-brain barrier. Annu Rev Pharmacol Toxicol. 2003;43:629–56.
188. De Gooijer MC, Kemper EM, Buil LCM, Ceren HC, Buckle T, Beijnen JH. ATP-binding cassette transporters restrict drug delivery and efficacy against brain tumors even when blood-brain barrier integrity is lost ll ATP-binding cassette transporters restrict drug delivery and efficacy against brain tumors even when bloo. Cell Reports Med. 2021;2:100184.
189. Demeule M, Régina A, Jodoin J, Laplante A, Dagenais C, Berthelet F, et al. Drug transport to the brain: Key roles for the efflux pump P-glycoprotein in the blood-brain barrier. Vascul Pharmacol. 2002;38:339–48.
190. Shapiro AB, Fox K, Lee P, Yang YD, Ling V. Functional intracellular P-glycoprotein. Int J Cancer. 1998;76:857–64.

191. Jodoin J, Demeule M, Fenart L, Cecchelli R, Farmer S, Linton KJ, et al. P-glycoprotein in blood-brain barrier endothelial cells: interaction and oligomerization with caveolins. J Neurochem. 2003;87:1010–23.
192. Schinkel AH, Wagenaar E, Mol CAAM, Van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest. 1996;97:2517–24.
193. Urquhart BL, Kim RB. Blood-brain barrier transporters and response to CNS-active drugs. Eur J Clin Pharmacol. 2009;65:1063–70.
194. Slot AJ, Wise DD, Deeley RG, Monks TJ, Cole SPC. Modulation of human Multidrug Resistance Protein (MRP) 1 (ABCC1) and MRP2 (ABCC2) transport activities by endogenous and exogenous glutathione-conjugated catechol metabolites. Drug Metab Dispos. 2008;36:552–60.
195. Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, et al. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: a review of the past decade. Cancer Lett. 2016;370:153–64.
196. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst. 2000;92:1295–302.
197. Zhong X, Liu MY, Sun XH, Wei MJ. Association between ABCB1 polymorphisms and haplotypes and Alzheimer's disease: a metaanalysis. Sci Rep. 2016;6:32708.
198. Fenart L, Buee-Scherrer V, Descamps L, Duhem C, Poullain MG. Inhibition of P-glycoprotein: rapid assessment of its implication in blood-brain barrier integrity and drug transport to the brain by an in vitro model of the blood-brain barrier. Pharm Res. 1998;15:993–1000.
199. Gruol DJ, Zee MC, Trotter J, Bourgeois S. Reversal of multidrug resistance by RU 4861. Cancer Res. 1994;54:3088–91.
200. Leggas M, Adachi M, Scheffer GL, Sun D, Wielinga P, Du G, et al. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. Mol Cell Biol. 2004;24:7612–21.
201. Bloise E, Ortiga-Carvalho TM, Reis FM, Lye SJ, Gibb W, Matthews SG. ATP-binding cassette transporters in reproduction: a new frontier. Hum Reprod Updat. 2016;22:164–81.
202. Russel FGM, Koenderink JB, Masereeuw R. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol Sci. 2008;29:200–7.
203. Potschka H, Fedrowitz M, Löscher W. Multidrug resistance protein MRP2 contributes to blood-brain barrier function and restricts antiepileptic drug activity. J Pharmacol Exp Ther. 2003;306:124–31.
204. Shawahna R, Uchida Y, Decleves X, Ohtsuki S, Yousif S, Dauchy S. Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. Mol Pharm. 2018;8:1332–41.
205. Grube M, Hagen P, Jedlitschky G. Neurosteroid transport in the brain: Role of ABC and SLC transporters. Front Pharmacol. 2018;9:354.
206. Jani M, Szabó P, Kis E, Molnár É, Glavinas H, Krajcsi P. Kinetic characterization of sulfasalazine transport by human ATP-binding cassette G2. Biol Pharm Bull. 2009;32:497–9.
207. Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann JM. Expression, up-regulation, and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. Cancer Res. 2004;64:3296–301.
208. Solár P, Zamani A, Kubíčková L, Dubový P, Joukal M. Choroid plexus and the blood-cerebrospinal fluid barrier in disease. Fluids Barriers CNS [Internet]. BioMed Central; 2020;17:1–29. Available from: https://doi.org/10.1186/s12987-020-00196-2.
209. Sun H, Dai H, Shaik N, Elmquist WF. Drug efflux transporters in the CNS. Adv Drug Deliv Rev. 2003;55:83–105.
210. Ghersi-Egea JF, Strazielle N, Catala M, Silva-Vargas V, Doetsch F, Engelhardt B. Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. Acta Neuropathol. 2018;135:337–61.
211. Hu C, Tao L, Cao X, Chen L. The solute carrier transporters and the brain: physiological and pharmacological implications. Asian J Pharm Sci. 2020;15:131–44.
212. Dvorak V, Wiedmer T, Ingles-Prieto A, Altermatt P, Batoulis H, Bärenz F, et al. An overview of cell-based assay platforms for the solute carrier family of transporters. Front Pharmacol. 2021;12:1–31.
213. Superti-Furga G, Lackner D, Wiedmer T, Ingles-Prieto A, Barbosa B, Girardi E, et al. The RESOLUTE consortium: unlocking SLC transporters for drug discovery. Nat Rev Drug Discov. 2020;19:429–30.
214. Bunney PE, Zink AN, Holm AA, Billington CJ, Kotz CM. Orexin activation counteracts decreases in nonexercise activity thermogenesis (NEAT) caused by high-fat diet. Physiol Behav. 2017;176:139–48.
215. Aykaç A, Şehirli AÖ. The Role of the SLC Transporters protein in the neurodegenerative disorders. Clin Psychopharmacol Neurosci. 2020;18:174–87.
216. Pan W, Kastin AJ. The blood-brain barrier: regulatory roles in wakefulness and sleep. Neuroscientist. 2017;23:124–36.
217. Cuddapah VA, Zhang SL, Sehgal A. Regulation of the blood–brain barrier by circadian rhythms and sleep. Trends Neurosci. 2019;42:500–10.
218. Friden PM. Receptor-mediated transport of therapeutics across the blood-brain barrier. Neurosurgery. 1994;35:294–8.
219. Anthony DP, Hegde M, Shetty SS, Rafic T, Mutalik S, Rao BSS. Targeting receptor-ligand chemistry for drug delivery across blood-brain barrier in brain diseases. Life Sci [Internet]. Elsevier Inc.; 2021;274:119326. Available from: https://doi.org/10.1016/j.lfs.2021.119326.
220. Jefferies WA, Brandon MR, Hunt SV, Williams AF, Gatter KC, Mason DY. Transferrin receptor on endothelium of brain capillaries. Nature. 1984;312:162–3.
221. Johnsen KB, Burkhart A, Thomsen LB, Andresen TL, Moos T. Targeting the transferrin receptor for brain drug delivery. Prog Neurobiol [Internet]. Elsevier; 2019;181:101665. Available from: https://doi.org/10.1016/j.pneurobio.2019.101665.
222. Duck KA, Connor JR. Iron uptake and transport across physiological barriers. Biometals. 2016;29:573–91.
223. Skjørringe T, Burkhart A, Johnsen KB, Moos T. Divalent metal transporter 1 (DMT1) in the brain: implications for a role in iron transport at the blood-brain barrier, and neuronal and glial pathology. Front Mol Neurosci. 2015;8:19.
224. Boado RJ, Pardridge WM. The Trojan horse liposome technology for nonviral Gene transfer across the blood-brain barrier. J Drug Deliv. 2011;296151.
225. Li H, Qian ZM. Transferrin/transferrin receptor-mediated drug delivery. Med Res Rev. 2002;22:225–50.
226. Jefferies WA, Brandon MR, Williams AF, Hunt SV. Analysis of lymphopoietic stem cells with a monoclonal antibody to the rat transferrin receptor. Immunology. 1985;54:333–41.
227. Pardridge WM. Tyrosine hydroxylase replacement in experimental Parkinson's disease with transvascular gene therapy. NeuroRx. 2005;2:129–38.
228. Saito Y, Buciak J, Yang J, Pardridge WM. Vector-mediated delivery of 125Ilabeled beta-amyloid peptide A beta 1–40 through the blood-brain barrier and binding to Alzheimer disease amyloid of the A beta 1–40/vector complex. Proc Natl Acad Sci. 1995;92:10227–31.
229. Song B-W, Vinters HV, Wu D, Pardridge WM. Enhanced neuroprotective effects of basic fibroblast growth factor in regional brain ischemia after conjugation to a blood-brain barrier delivery vector. J Pharmacol Exp Ther. 2002;301:605–10.

230. Couch JA, Yu YJ, Zhang Y, Tarrant JM, Fuji RN, Meilandt WJ, et al. Addressing safety liabilities of TfR bispecific antibodies that cross the blood-brain barrier. Sci Transl Med. 2013;5:1–12.
231. Israel LL, Braubach O, Galstyan A, Chiechi A, Shatalova ES, Grodzinski Z, Ding H, Black KL, Ljubimova JY, Holler E. A combination of tri-leucine and angiopep-2 drives a polyanionic polymalic acid nanodrug platform across the bloodbrain barrier. ACS Nano. 2019;13:1253–71.
232. Friden PM, Walus LR, Musso GF, Taylor MA, Malfroy B, Starzyk RM. Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood brain barrier. Proc Natl Acad Sci U S A. 1991;88:4771–5.
233. Friden PM, Walus LR, Watson P, Doctrow SR, Kozarich JW, Bäckman C, Bergman H, Hoffer B, Bloom F, Granholm AC. Blood-brain barrier penetration and in vivo activity of an NGF conjugate. Science. 1993;259:373–7.
234. Granholm AC, Bäckman C, Bloom F, Ebendal T, Gerhardt GA, Hoffer B, Mackerlova L, Olson L, Söderström S, Walus LR. NGF and anti-transferrin receptor antibody conjugate: short and long-term effects on survival of cholinergic neurons in intraocular septal transplants. J Pharmacol Exp Ther. 1994;268:448–59.
235. Charles V, Mufson EJ, Friden PM, Bartus RT, Kordower JH. Atrophy of cholinergic basal forebrain neurons following excitotoxic cortical lesions is reversed by intravenous administration of an NGF conjugate. Brain Res. 1996;728:193–203.
236. Pardridge WM, Wu D, Sakane T. Combined use of carboxyl-directed protein pegylation and vector-mediated blood-brain barrier drug delivery system optimizes brain uptake of brain-derived neurotrophic factor following intravenous administration. Pharm Res. 1998;15:576–82.
237. Deguchi Y, Kurihara A, Pardridge W. Retention of biologic activity of human epidermal growth factor following conjugation to a blood-brain barrier drug delivery vector via an extended poly(ethylene glycol) linker. Bioconjug Chem. 1999;10:32–7.
238. Bickel U, Yoshikawa T, Landaw EM, Faull KF, Pardridge WM. Pharmacologic effects in vivo in brain by vector-mediated peptide drug delivery. Proc Natl Acad Sci U S A. 1993;90:2618–22.
239. Xia C-F, Zhang Y, Zhang Y, Boado RJ, Pardridge WM. Intravenous siRNA of brain cancer with receptor targeting and avidin-biotin technology. Pharm Res. 2007;24:2309–16.
240. Pardridge WM, Boado RJ, Kang YS. Vector-mediated delivery of a polyamide ("peptide") nucleic acid analogue through the blood-brain barrier in vivo. Proc Natl Acad Sci U S A. 1995;92:5592–6.
241. Moos T, Morgan EH. Restricted transport of anti-transferrin receptor antibody (OX26) through the blood-brain barrier in the rat. J Neurochem. 2001;79:119–29.
242. Cabezón I, Augé E, Bosch M, Beckett AJ, Prior IA, Pelegrí C, Vilaplana J. Serial block-face scanning electron microscopy applied to study the trafficking of 8D3-coated gold nanoparticles at the blood-brain barrier. Histochem Cell Biol. 2017;36:437.
243. Manich G, Cabezón I, del Valle J, Duran-Vilaregut J, Camins A, Pallàs M, Pelegri C, Vilaplana J. Study of the transcytosis of an anti-transferrin receptor antibody with a Fab' cargo across the blood-brain barrier in mice. Eur J Pharm Sci. 2013;49:556–64.
244. Huwyler J, Cerletti A, Fricker G, Eberle AN, Drewe J. By-passing of P-glycoprotein using immunoliposomes. J Drug Target. 2002;10:73–9.
245. Zhang Y, Schlachetzki F, Zhang Y-F, Boado RJ, Pardridge WM. Normalization of striatal tyrosine hydroxylase and reversal of motor impairment in experimental parkinsonism with intravenous nonviral gene therapy and a brainspecific promoter. Hum Gene Ther. 2004;15:339–50.
246. Bommana MM, Kirthivasan B, Squillante E. In vivo brain microdialysis to evaluate FITC-dextran encapsulated immunopegylated nanoparticles. Drug Deliv. 2012;19:298–306.
247. Loureiro JA, Gomes B, Fricker G, Coelho MAN, Rocha S, Pereira MC. Cellular uptake of PLGA nanoparticles targeted with anti-amyloid and anti-transferrin receptor antibodies for Alzheimer's disease treatment. Colloids Surf B Biointerfaces. 2016;145:8–13.
248. Candela P, Saint-Pol J, Kuntz M, Boucau MC, Lamartiniere Y, Gosselet F, Fenart L. In vitro discrimination of the role of LRP1 at the BBB cellular level: focus on brain capillary endothelial cells and brain pericytes. Brain Res. 2015;1594:15–26.
249. Dehouck B, Fenart L, Dehouck MP, Pierce A, Torpier G, Cecchelli R. A new function for the LDL receptor: transcytosis of LDL across the blood-brain barrier. J Cell Biol. 1997;138:877–89.
250. Wang P, Liu Y, Shang X, Xue Y. CRM197-induced blood-brain barrier permeability increase is mediated by upregulation of caveolin-1 protein. J Mol Neurosci. 2011;43:485–92.
251. Lesley J, Schulte R, Woods J. Modulation of transferrin receptor expression and function by anti-transferrin receptor antibodies and antibody fragments. Exp Cell Res. 1989;182:215–33.
252. Lee HJ, Engelhardt B, Lesley J, Bickel U, Pardridge WM. Targeting rat anti-mouse transferrin receptor monoclonal antibodies through blood-brain barrier in mouse. J Pharmacol Exp Ther. 2000;292:1048–52.
253. Huang R, Ke W, Han L, Liu Y, Shao K, Jiang C, et al. Lactoferrin-modified nanoparticles could mediate efficient gene delivery to the brain in vivo. Brain Res Bull [Internet]. 2010;81:600–4. Available from: https://doi.org/10.1016/j.brainresbull.2009.12.008.
254. Demeule M, Regina A, Ché C, Poirier J, Nguyen T, Gabathuler R, et al. Identification and design of peptides as a new drug delivery system for the brain. J Pharmacol Exp Ther [Internet]. 2008;324:1064–72. Available from: https://doi.org/10.1124/jpet.107.131318.
255. Tamaru M, Akita H, Fujiwara T, Kajimoto K, Harashima H. Leptin-derived peptide, a targeting ligand for mouse brain-derived endothelial cells via macropinocytosis. Biochem Biophys Res Commun [Internet]. 2010;394:587–92. Available from: https://doi.org/10.1016/j.bbrc.2010.03.024.
256. Vinante F, Rigo A. Heparin-binding epidermal growth factor-like growth factor/diphtheria toxin receptor in normal and neoplastic hematopoiesis. Toxins (Basel). 2013;5:1180–201.
257. Gaillard PJ, Brink A, de Boer AG. Diphtheria toxin receptor-targeted brain drug delivery. Int Congr Ser. 2005;1277:185–98.
258. Wang P, Xue Y, Shang X, Liu Y. Diphtheria toxin mutant CRM197-mediated transcytosis across blood-brain barrier in vitro. Cell Mol Neurobiol. 2010;30:717–25.
259. Tosi G, Vilella A, Veratti P, Belletti D, Pederzoli F, Ruozi B, et al. Exploiting bacterial pathways for BBB crossing with PLGA nanoparticles modified with a mutated form of diphtheria toxin (CRM197): In vivo experiments. Mol Pharm. 2015;12:3672–84.
260. Osada T, Gu YH, Kanazawa M, Tsubota Y, Hawkins BT, Spatz M, et al. Interendothelial claudin-5 expression depends on cerebral endothelial cell-matrix adhesion by B 1-integrins. J Cereb Blood Flow Metab. 2011;31:1972–85.
261. Santulli G, Lewis D, des Georges A, Marks AR, Frank J. Ryanodine receptor structure and function in health and disease. Subcell Biochem. 2018;87:329–52.
262. Goti D, Hrzenjak A, Levak-Frank S, Frank S, Van Der Westhuyzen DR, Malle E, et al. Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. J Neurochem. 2001;76:498–508.
263. Bareford LM, Swaan PW. Endocytic mechanisms for targeted drug delivery. Adv Drug Deliv Rev. 2007;59:748–58.
264. Liu Y, Huang R, Han L, Ke W, Shao K, Ye L, et al. Brain-targeting gene delivery and cellular internalization mechanisms for modified rabies virus glycoprotein RVG29 nanoparticles. Biomaterials. 2009;30:4195–202.
265. Lajoie JM, Shusta EV. Targeting receptor-mediated transport for delivery of biologics across the blood-brain barrier. Annu Rev Pharmacol Toxicol. 2015;55:613–31.

266. Abulrob A, Sprong H, En Henegouwen PVB, Stanimirovic D. The blood-brain barrier transmigrating single domain antibody: mechanisms of transport and antigenic epitopes in human brain endothelial cells. J Neurochem. 2005;95:1201–14.
267. Bien-Ly N, Yu YJ, Bumbaca D, Elstrott J, Boswell CA, Zhang Y, et al. Transferrin receptor (TfR) trafficking determines brain uptake of TfR antibody affinity variants. J Exp Med. 2014;211:233–44.
268. Sade H, Baumgartner C, Hugenmatter A, Moessner E, Freskgård PO, Niewoehner J. A human blood-brain barrier transcytosis assay reveals antibody transcytosis influenced by pH-dependent receptor binding. PLoS ONE. 2014;9:e96340.
269. Zuchero YJY, Chen X, Bien-Ly N, Bumbaca D, Tong RK, Gao X, et al. Discovery of novel blood-brain barrier targets to enhance brain uptake of therapeutic antibodies. Neuron. 2016;89:70–82.
270. Carvey PM, Hendey B, Monahan AJ. The blood-brain barrier in neurodegenerative disease: a rhetorical perspective. J Neurochem. 2009;111:291–314.
271. Avnir Y, Turjeman K, Tulchinsky D, Sigal A, Kizelsztein P, Tzemach D, et al. Fabrication principles and their contribution to the superior in vivo therapeutic efficacy of nano-liposomes remote loaded with glucocorticoids. PLoS ONE. 2011;6:e25721.
272. Turjeman K, Bavli Y, Kizelsztein P, Schilt Y, Allon N, Katzir TB, et al. Nano-drugs based on nano sterically stabilized liposomes for the treatment of inflammatory neurodegenerative diseases. PLoS ONE. 2015;10:e0130442.
273. Sarantopoulos J, Gabrail NY, Moulder SL, Brenner AJ, Smith CL, Bouchard D, et al. ANG1005: Results of a phase I study in patients with advanced solid tumors and brain metastases. J Clin Oncol. 2010;28:2556–2556.
274. Lu Q, Cai X, Zhang X, Li S, Song Y, Du D, Dutta P, Lin Y. Synthetic polymer nanoparticles functionalized with different ligands for receptor-mediated transcytosis across the blood-brain barrier. ACS Appl Biomat. 2018;1:1687–94.
275. Lakkadwala S, Singh J. Co-delivery of doxorubicin and erlotinib through liposomal nanoparticles for glioblastoma tumor regression using an in vitro brain tumor model. Colloids Surf B Biointerfaces. 2019;173:27–35.
276. Lakkadwala S, dos Santos Rodrigues B, Sun C, Singh J. Biodistribution of TAT or QLPVM coupled to receptor targeted liposomes for delivery of anticancer therapeutics to brain in vitro and in vivo. Nanomed Nanotechnol Biol Med. 2020;23:102112.
277. Bouzinab K, Summers HS, Stevens MFG, Moody CJ, Thomas NR, Gershkovich P, et al. Delivery of temozolomide and N3-propargyl analog to brain tumors using an apoferritin nanocage. ACS Appl Mater Interfaces. 2020;12:12609–17.
278. Pardridge WM. Human blood? Brain barrier insulin receptor biologic drug development for the CNS view project. J Neurochem. 1985.
279. Coloma MJ, Lee HJ, Kurihara A, Landaw EM, Boado RJ, Morrison SL, Pardridge WM. Transport across the primate blood-brain barrier of a genetically engineered chimeric monoclonal antibody to the human insulin receptor. Pharm Res. 2000;17:266–74.