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THE ROLE OF DRUG TRANSPORTERS AT THE BLOOD-BRAIN BARRIER

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■ **Abstract** The blood-brain barrier (BBB) is a dynamic interface between the blood and the brain. It eliminates (toxic) substances from the endothelial compartment and supplies the brain with nutrients and other (endogenous) compounds. It can be considered as an organ protecting the brain and regulating its homeostasis. Until now, many transport systems have been discovered that play an important role in maintaining BBB integrity and brain homeostasis. In this review, we focus on the role of carrier- and receptor-mediated transport systems (CMT, RMT) at the BBB. These include CMT systems, such as P-glycoprotein, multidrug-resistance proteins 1–7, nucleoside transporters, organic anion transporters, and large amino-acid transporters; RMT systems, such as the transferrin-1 and -2 receptors; and the scavenger receptors SB-AI and SB-BI.

INTRODUCTION AND SCOPE

Ehrlich (1) and Goldman (2) were the first to observe the existence of the blood-brain barrier (BBB) after the injection of the hydrophilic compound trypan blue in a rat did not distribute into and out of the brain. It is now known that the cerebral capillary regulates the influx and efflux of biologically important molecules both by preventing passive hydrophilic diffusion and by providing transport processes whose activity can be regulated in accordance with the metabolic and homeostatic requirements of the brain.

Drug transport to the brain depends on various parameters. The amount of drug available for transport across the BBB depends upon its systemic pharmacokinetics [represented by absorption, distribution, metabolism, and elimination (ADME); see also Figure 1]. For drugs that can easily pass the BBB, blood flow is a limiting factor, whereas for other drugs, BBB-permeability is restrictive. In addition, the cardiac output to the brain seems not to be the main determinant for blood flow, but rather the local blood flow and the capillary flow area. In vivo capillary flow was

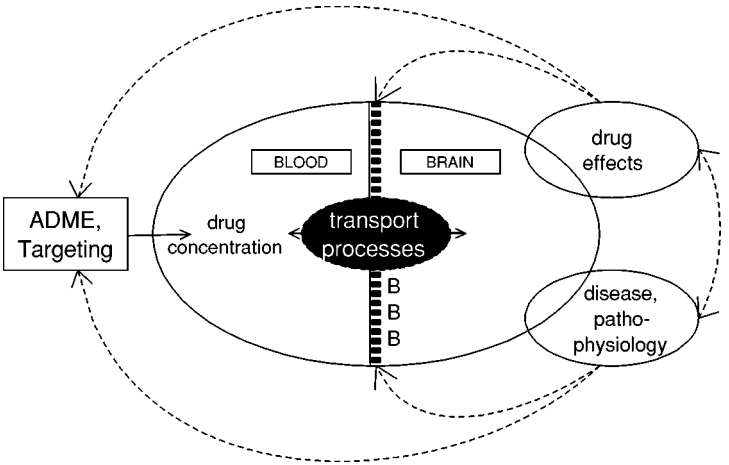

Figure 1 An illustration of the BBB as an organ protecting and regulating the homeostasis of the brain. In addition, the influence of pharmacokinetics (ADME) and drug targeting on the amount of drug available for transport to the brain and the influence of drug effects (pharmacodynamics) and disease/pathophysiology on BBB functionality and integrity is illustrated. The broken lines indicate the influence of drug effects and disease/pathophysiology on the systemic pharmacokinetics (ADME, targeting) and the various transport processes at the BBB [modified from (154)].

shown to be very low because the capillary pressure was approximately 17 mm Hg and not continuous (3). Another factor, protein binding, controls the free (unbound) fraction of drugs/compounds and their distribution in blood that both influence the amount that will be able to pass the BBB. Presently, it is assumed that only the free fraction of drug in plasma/blood is able to pass the BBB. This view may be too limited because many binding proteins (albumin, alpha-acid-glycoprotein, globulins, HDL (high-density lipoprotein), LDL (low-density lipoprotein), insulin-like growth factor binding proteins, etc.) present in plasma could "donate" ligands to the BBB.

Systemic metabolism of drugs occurs mainly in the liver; however, metabolism can also occur during BBB transport because many enzymes are present in the brain capillaries (4). These can transform drugs, before entering the central nervous system (CNS), into metabolites that can be more or less effective or even toxic.

The physico-chemical characteristics of drugs (e.g., hydrophilicity, lipophilicity, hydrogen bonding potential) largely determine the passive transport of drugs across the BBB. This comprises hydrophilic paracellular and lipophilic transcellular transport. Passive hydrophilic transport is mainly restricted by the tight junctions of the BBB endothelial cells. This paracellular permeability is, next to size (5), further dependent on the charge of the molecules and the possibility to form

hydrogen bonds. Passive transcellular processes are mainly dependent on the log-P (log-partition coefficient) or log-D (log-P at pH 7.4) values and their hydrogen bonding potential. There is a linear relationship between log-P and BBB transport in vivo (6). In addition, passive drug transport can be predicted in silico by application of the dynamic polar surface area method, which shows a very good correlation between the polar surface of a molecule and its in vitro transport across a Caco-2 epithelial monolayer (7). Similarly, data from our laboratory showed that the in vitro, in situ, and in vivo BBB transport of adenosine analogues correlate well with their calculated BBB permeability based on hydrogen donor and hydrogen acceptor properties and the lipophilicity of the compounds.

The physical barrier properties of the BBB are related to its narrow tight junctions, the absence of intercellular clefts, minor pinocytotic activity, a nonfenestrated endothelium, a continuous basement membrane, many mitochondria (9), a high electrical resistance (1500–2000 Ohm cm^2) (10), and anionic sites at the luminal membrane. Several of these BBB characteristics are induced by astrocytes (11). However, in small parts of the brain, particularly the circumventricular organs (CVOs), the BBB is physically leaky due to a fenestrated endothelium, but the contribution of drug transport to the brain from these regions is rather limited. Because of its physical barrier properties and to maintain BBB homeostasis, various transport processes, including influx and efflux transporters, operate at the BBB to supply and eliminate substances to and from the endothelial compartment, and subsequently, the brain. These include fluid phase–mediated- (FMT), adsorptive-mediated- (AMT), receptor-mediated- (RMT), and carrier-mediated transport (CMT). AMT depends on the negative charge of the membrane and is therefore rather unspecific and suited for cationic or cationized compounds (see Figure 2). FMT is nonsaturable and also unspecific, and the amount of compound that can be internalized depends upon its extracellular concentration. Both processes occur to a very low extent at a healthy BBB.

CMT occurs by membrane-fixed transporters that transport substances of relatively small size. In contrast, RMT occurs by receptors that are internalized with their ligand(s). These systems can internalize larger substances/particles. Both can be saturated or inhibited, competitively or noncompetitively. In addition, CMT and RMT are selective or specific processes and can be used to target drugs/substances to the BBB/brain. However, internalization or endocytosis is only the first step in the transcytosis of drugs/substances across the BBB endothelial cells, resulting in transport to the brain. As stated earlier, metabolism, but also degradation in the lysosomes can occur, reducing the amount of drug that reaches the brain.

All of these features, including their regulatory systems, provide the physiological basis to understand changed BBB functionality and permeability and therefore the transport of compounds into and out of the brain. Moreover, these influx and efflux systems also maintain BBB functionality by transporting substances in and out of the endothelial compartment. Therefore, the BBB can be considered an

organ that protects the brain and regulates its homeostasis. In this review, we focus on the role of transporters at the BBB. Particularly, we discuss in detail the efflux carriers P-glycoprotein (Pgp) and to a smaller extent the multidrug-resistance proteins (MRPs). Further, we discuss other CMT systems, including the nucleoside-, organic anion-, and organic anion polypeptide-transporter, the large amino acid carrier, and the RMT systems, including the transferrin- and the scavenger receptors. For an overview of transporters at brain barriers, the reader is referred to recent reviews (12–15).

BLOOD-BRAIN BARRIER HOMEOSTASIS AND DISEASE

It has been well recognized that diseases and pathophysiology influence BBB functionality and/or permeability. BBB permeability is changed under various disease conditions, e.g., multiple sclerosis (16), Alzheimer's disease (17), AIDS (18), AIDS-related dementia (19), inflammation-like encephalitis and meningitis (20), and hypertension and seizures, but also in psychiatric disorders (21). These diseases can influence BBB permeability directly or indirectly; directly via changed paracellular permeability, indirectly via changed functionality of BBB transport processes. This can occur at therapeutic concentrations, but also following accumulation of these drugs in BBB endothelial cells when transport systems are inhibited or become less effective, which can result in toxic intracellular concentrations. Ultimately, these changes can lead to CNS disturbances. Therefore, drugs developed to treat these diseases can also influence BBB permeability directly or indirectly. This is schematically illustrated in Figure 1.

There are various examples of drugs/compounds and diseases that influence BBB functionality. The influence of BBB pathology and the influence of disease mediators, such as LPS, NO, radical oxygen species/radical nitrogen species (ROS/RNS), etc., on BBB permeability is well-known, particularly with respect to paracellular permeability (22). Further, disease can up- or downregulate active transcellular transport systems (transporters and transcytosis mechanisms). Lesser known is the influence of the pharmacodynamic effects of drugs at the BBB, e.g., glucocorticoids and interferons that are able to make the BBB tighter (23, 24).

Very interesting experiments have demonstrated the influence of beta-amyloid$_{1\text{-}42}$ on BBB functionality (25). Intracarotid infusions of beta-amyloid$_{1\text{-}42}$ increase BBB permeability in rats. A dose-dependent increase in albumin-bound Evans blue is observed following administration of this peptide. In addition, histological studies indicated an almost complete disappearance of lectin binding sites at the affected endothelial cells. These results indicate that changes occur in the BBB endothelium, suggesting a role in the development of brain pathologies that are associated with Alzheimer's disease. These data are supported by earlier findings that aortic and pulmonary endothelial cells were severely damaged following exposure to beta-amyloid$_{1\text{-}42}$ (26).

Other experiments reveal the influence of apolipoprotein (apo) E on maintaining the integrity of the BBB in rats (27). Apo E in the brain is synthesized by astrocytes, and it was shown that apo E (E_2, E_3, and E_4) plays an important role in the development of Alzheimer's disease (28). Further, apo E knockout mice develop severe artheriosclerosis that is enhanced by a high-fat diet (29). Here, it was demonstrated that apo E3–Leiden mice, which develop severe artheriosclerosis that is enhanced by a high-fat diet, did not show signs of BBB disturbances, whereas apo-E-knockout mice did when put on a high-fat high-cholesterol diet (27). The results indicate a severe extravasation of IgG in the hippocampus and cerebellum of apo E–knockout mice and not in C57B1/6 control mice, which points to increased BBB transport. In addition, the transport of the vascular marker fluorescein into the brain of male and female knockout mice showed a tendency to increase compared to C57b1/6 control mice, which points to increased paracellular BBB transport. These data were supported by behavioral analysis, indicating that apo E–knockout mice were less efficient in acquiring the spatial Morris water maze task compared to C57B1/6 mice. These interesting observations relate cognitive functions with BBB functionality. It is hypothesized that apo E plays an important role in maintaining the integrity of the BBB and affects neurodegenerative processes as seen in Alzheimer's disease.

These experiments illustrate the importance of transport processes at the BBB to maintain CNS homeostasis for optimal CNS performance. However, it still has to be revealed if BBB disfunctionality will be the cause or the result of CNS diseases.

IN VITRO AND IN VIVO METHODS TO STUDY BLOOD-BRAIN BARRIER TRANSPORT

Research on drug transport across the BBB and its functionality has been very much enhanced by the availability of in vitro BBB endothelial/astrocyte (co)-culture systems. The use of such systems allows a detailed investigation of BBB-related phenomena at the (sub)-cellular level and in the absence of feedback systems from the rest of the body. This makes it much easier to study in vitro BBB transport and BBB functionality by (pharmacological) intervention techniques, such as the application of receptor agonists and antagonists, blockers of transporters and enzymes, antisense and (anti)gene approaches, and the influence of disease. Recently, BBB (co)-culture systems have been reviewed and discussed with respect to their use in BBB-related research (30, 31).

Various methods have been developed to estimate in vivo BBB drug transport also. The applicability of these methods depends on their sensitivity and selectivity to measure drug concentrations in the brain, the estimation of local concentrations in the brain (spatial resolution), and the measurement of single-time concentrations versus concentration-time profiles (time resolution). Because an extended discussion is beyond the scope of this review, the reader is referred to the literature (32).

TRANSPORT PROCESSES AT THE BLOOD-BRAIN BARRIER

Carrier Mediated Transport

Transporters comprise carriers and receptors that are located in the plasma membrane of the endothelial cells of the BBB. Carriers are membrane-restricted systems suited to generally transport compounds with a rather fixed size and a molecular mass smaller than 500–600 Da (33). Most of these systems are ATP-driven and therefore possess at least one intracellular ATP-binding domain. Others are equilibrative systems and do not require ATP. The activity of these transporters is temperature sensitive and they can be saturated at higher concentrations of ligands. Their activity can be influenced by competitive and noncompetitive inhibitors and by interfering with their phosphorylation by protein kinases.

Receptor Mediated Transport

Receptors may also be located in the plasma membrane of the endothelial cells of the BBB, but are not restricted to this location. They can be internalized and transported via the early endosome to the lysosomes or even transcytosed and shuttled back to the plasma membrane again. Internalization occurs via an endocytotic process. Endocytosis is used in this context to indicate vesicular transport pathways in eukaryotic cells to internalize extracellular fluid and particles (<500 nm) as well as plasma membrane molecules (34). Endocytosis may be very fast; in some cultured mammalian cells, 50% of the entire cell surface may be internalized every hour (35).

With receptor-mediated endocytosis, the uptake of particles or ligands is saturable because it is dependent upon the extracellular availability of receptors. Subsequently, following binding, the ligand-receptor complex is internalized. This process requires energy and is also temperature sensitive. In addition, the internalization process is time dependent. The half-life time for internalization of several proteins by coated vesicles under optimal conditions varies from 2–5 min (34). Further, the rate of internalization may be dependent on ligand binding (regulated endocytosis), whereas other receptors may be internalized equally fast with or without ligand [constitutive endocytosis; (36)]. In contrast to carriers, receptors are able to internalize relatively large compounds and systems and are therefore more suited for targeted drug delivery (peptides, proteins, etc.) to the brain (37).

Endocytosis

Endocytosis can occur via two distinct pathways, e.g., via coated or noncoated invaginations in the membrane of cells. Coated invaginations, or coated-pits, have shown the presence of clathrin, and the influence of adaptor-protein 2 (AP-2) complexes and dynamin (a GTPase required for budding of clathrin coated vesicles) as major components of the coated-pits have been demonstrated (34). The AP-2

adaptor complex plays an important role in the recognition of internalization motifs of extracellular receptors. Tyrosine-based motifs, such as NPXY and YXXO (the characters indicate single amino acids where X is any amino acid and O is a bulky hydrophobic amino acid), and the dileucine (LL) motif have been recognized (34). Various receptors, like the low-density lipoprotein, the transferrin-, and the mannose 6-phophate receptors, are internalized via these coated-pits.

Noncoated omega, smooth-coated, or flask-shaped invaginations (35) were initially discovered by Palade et al. (38). They are called caveolae and have a maximal diameter of 70 nm. They start from the plasmalemma and have a neck-like structure. By electron microscopy, caveolae can be distinguished from clathrin-coated pits because they are not associated with an electron-dense cytoplasmic coat. Caveolae are abundantly present in endothelial and smooth muscle cells, but are also present in epithelial cells, adipocytes, fibroblasts, type 1 pneumocytes, and striated muscle cells (39). Caveolae are coated with proteins that belong to the family of caveolins [caveolin-1(alpha,1beta), caveolin-2(alpha, beta, gamma), and caveolin-3; (39)], but other proteins, such as flotillin-1, MAL1, and MEC-2/stomatin proteins, may also be involved in caveolae formation (40).

The function of caveolae includes the transport of molecules across these cells, but recently, it was shown that caveolae are also involved in potocytosis (the internalization of small molecules without the merging of an endocytotic vesicle with endosomes), signal transduction regulation, and cholesterol transport (41).

In conclusion, it may be clear that RMT and CMT are important processes for the influx and efflux of substances to and from the BBB endothelial compartment. Changed activity of these processes due to disease, for example, can have serious consequences for functionality and integrity of the BBB. On one hand, this can result in increased para- and transcellular BBB permeability, and therefore, in changed CNS homeostasis. Ultimately, this can lead to CNS diseases, e.g., Alzheimer's or other neurodegenerative diseases (25–27). On the other hand, this offers opportunities for site-specific or targeted drug delivery to the brain when transport processes are selectively upregulated under disease conditions.

ROLE OF TRANSPORT PROCESSES AT THE BLOOD-BRAIN BARRIER: CARRIER-MEDIATED TRANSPORT OF DRUGS

Pgp Efflux Pump

One of the most important efflux transporters identified at the BBB is the Pgp efflux system. This system is discussed in more detail because its presence has been demonstrated at the luminal site of the BBB endothelium (42) and it has been extensively studied in past decades. The Pgp efflux system is responsible for the occurrence of multidrug resistance (MDR), and today, Pgp is considered as an amphipatic cationic efflux pump.

Pgp is a 170-kDa membrane-fixed glycoprotein and comprises two almost identical halves within 12 alpha-helical transmembrane-spanning domains and two intracellular ATP-binding sites. It belongs to the ABC (ATP-binding cassette) superfamily, which consists of more than 30 families transporting a tremendous variety of substrates.

In humans, the MDR1 and the MDR2 genes encode for the two different isotypes of Pgp (43). The MDR1-Pgp is mainly found in the apical membrane of epithelial tissues from the intestine, kidney, pancreas, and adrenal gland. Further, it has been found in the endothelium from the endocervix, endometrium, esophagus, glomeruli, intestine, lung, lymph nodes, myometrium, placental trophoblasts, ovarian cortex, papillary dermis, prostate, spleen, stomach, testes, blood-inner ear, and the BBB (42, 44, 45). Recently, Pgp was demonstrated to be partially localized in the caveolae of resistant (CH^RC5) cells and of drug-sensitive Chinese hamster ovary (CHO) (AuxB1) cells. A similar localization of Pgp was found in caveolae of rat brain capillary cells (46).

In rodents, there are three Pgp genes encoding for the mdr1a-, the mdr1b-, and the mdr2-Pgp (47). The mdr1a- and the mdr1b-gene products fulfill the same function as the MDR1-gene product in humans.

MDR2- and mdr2-Pgp do not play an important role in the transport of drugs. They are abundantly expressed in the liver and their function has been demonstrated by the transport of phospholipids across the canicular membranes in hepatocytes into the bile (48).

Physiological Role of Pgp

It has been postulated that Pgp acts as a so-called vacuum cleaner (49), moving compounds from the lipid bilayer into the extracellular space. A second hypothesis has been postulated where the transporter acts as a flippase (50), either moving the substrate from the inner to the outer leaflet of the membrane or locally altering membrane lipid composition such that the substrate detaches. These mechanisms support the observation that Pgp effluxes amphipatic peptides, proteins lacking signal sequences, or lipid-modified proteins from biological membranes (51).

The location of Pgp, particularly at blood-tissue and air-tissue interfaces, and its broad range of substrates, indicates that it limits the influx and diffusion of compounds and, subsequently, the exposure of cells to high (toxic) concentrations of compounds. In addition, it has been suggested that Pgp has several physiological functions in mammals (52). There is also evidence that Pgp transports steroid hormones. Therefore, the increased expression of Pgp during pregnancy in the placenta, embryo, and uterus may explain the need to protect the fetal tissue against these hormones (53, 54). Furthermore, Karssen et al. (55) demonstrated in mdr1a(+/+) and -(−/−) mice that Pgp was involved in limiting the access of the naturally occurring glucocorticoid cortisol rather than corticosterone to the mouse as well as human brain, particularly to the hippocampal area. In addition, similar

data were found for the glucocorticoid dexamethason (56). Because glucocorticoids influence behavior, it was suggested that Pgp may play an important role in the regulation of the behavioral response of glucocorticoids in the hippocampus.

The hypothesis that Pgp regulates volume-activated chloride channels was developed by the observation that Pgp was highly related to the cystic fibrosis transmembrane regulator protein (CFTR). CFTR belongs, like Pgp, to the ABC superfamily of transporters and is a plasma membrane chloride channel that is dysfunctional in cystic fibrosis (50). However, the role of Pgp in volume-regulatory processes has been controversial, and a current hypothesis is that Pgp does not have channel activity itself, but it may regulate swelling-induced anion channels (57).

It has been suggested that Pgp plays a role in the transport of prenylcysteinmethyl esters or cholesterol (58). In addition, esterification of cholesterol and triacylglycerol-rich lipoprotein secretion was inhibited by inhibitors of Pgp (59), whereas cholesterol seemed to be transported by Pgp also (60).

Recently, it was demonstrated that beta-amyloid$_{1\text{-}42}$ was transported by Pgp (61). Beta-amyloid$_{1\text{-}42}$ is an amphipatic peptide comprised of 28 hydrophilic and 12–14 hydrophobic amino acids. These peptides are rapidly released from both neuronal and nonneuronal cells; however, the sequence of the 12–14 hydrophobic amino acids is the reason that the peptide remains associated with the membrane following gamma-secretase cleavage. Therefore, it has been postulated that transport systems are required to efflux these peptides. In vitro binding studies show that addition of synthetic human beta-amyloid$_{1\text{-}40}$ and beta-amyloid$_{1\text{-}42}$ peptides to hamster mdr1-enriched vesicles results in saturated quenching. This suggests that both peptides interact directly with the transporter. Inhibition studies with the MDR1 inhibitors RU-486 and RU-49953 in MDR1-transfected cells reduced the secretion of the peptides. It was concluded that these data were strongly suggestive for Pgp-mediated efflux of beta-amyloid peptides.

In conclusion, Pgp is involved in the regulation of various physiological processes. Furthermore, the fact that mdr1a(−/−) and mdr1a/1b(−/−) mice appear normal suggests that Pgp does not have an essential role in life, although it should be taken into account that due to the knockout of mdr1a and mdr1b genes, Pgp function may be compensated by other transporters.

Pgp and Pharmacodynamic Effects of Drugs in the Brain

Important evidence for the role of Pgp at the BBB was obtained from experiments with mdr1a(−/−) and mdr1a/1b(−/−) mice. The significantly higher accumulation of several drugs in the brains of these mice in comparison to most other tissues and plasma demonstrated its important role (62). In addition, increased accumulation of these drugs in various tissues can affect their pharmacodynamics (63). This is best illustrated by centrally acting drugs. Morphine is often used as a narcotic analgesic for the treatment of pain. It acts at the opioid receptors within the CNS at both the spinal and supraspinal levels. In vitro and in vivo studies have

demonstrated that morphine is a weak Pgp substrate (64). Pgp influence on the pharmacodynamics of morphine was studied in mdr1a(−/−) and wild-type mice (65). The tail-flick response to radiant heat was taken as the pharmacodynamical endpoint to determine the antinociceptive effect. Morphine was administered subcutaneously to both mdr1a(−/−) and wild-type mice and it was found that antinociception was indeed increased in the mdr1a(−/−) mice. The ED_{50} of morphine was more than twofold lower in mdr1a(−/−) mice (3.8 +/− 0.2 mg/kg) compared to FVB (wild-type) mice (8.8 +/− 0.2 mg/kg), whereas EC_{50} in brain tissue was similar. Pgp inhibition in wild-type mice with R-verapamil resulted in an antinociceptive effect similar to that in mdr1a(−/−) mice. A comparable study was performed in rats, where GF120918 was used as a Pgp inhibitor (66). Both the antinociceptive effect was measured and the concentrations of morphine and its main metabolite morphine-3-glucuronide (M3G) in blood and brain extracellular fluid (ECF) were measured by intracerebral microdialysis. Inhibition of Pgp resulted in increased concentrations of morphine and M3G in the brain, whereas in the blood, only M3G concentrations were changed. The pharmacodynamic effect of morphine was increased in the presence of GF120918 and could be described using a pharmacokinetic/pharmacodynamic model based on morphine concentrations in the ECF.

It was suggested that pharmacoresistance for the antiepileptic drug phenytoin was caused by Pgp at the BBB (67). Phenytoin concentrations in the ECF of the cerebral cortex or rat brain were measured by microdialysis. Pgp inhibitors, such as sodium cyanide, verapamil, and PSC 8333, were directly administered by a 15–60 min infusion via a microdialysis probe in the right frontal cortex before intraperitoneal administration of phenytoin. Phenytoin concentrations in the ECF were significantly enhanced by Pgp inhibitors, indicating that Pgp limited the distribution of phenytoin into the brain. Interestingly, a similar effect was found for Cremophor, the vehicle used to administer the PSC 833. In addition, based on in vitro experiments in bovine BCEC and a Pgp-overexpressing cell line (MCF-7/Adr) and in vivo experiments in mdr1a(+/+) and (−/−) mice, similar results were found for some enaminone anticonvulsants (68).

The influence of Pgp on the therapeutic effects of drugs in the CNS is particularly illustrated by the effect of Pgp at the BBB in limiting the treatment of brain tumors. The presence of Pgp in tumors causes MDR, but Pgp at the BBB is also responsible for MDR in the case of brain tumors. Several anticancer drugs are Pgp substrates and poorly pass the BBB. Consequently, these drugs will not reach tumors in sufficient concentrations. Using Pgp inhibitors in cancer therapy can therefore be beneficial in two ways. First, the pharmacokinetics of the anticancer drugs can change; particularly, CNS drug concentrations can increase. Second, the intracellular drug concentration in brain tumors can increase (provided that the inhibitor also distributes to the brain tumor).

An increase in in vitro BBB permeability was observed following concomitant administration of the Pgp substrate vinblastine and the Pgp inhibitor PSC 833 (69, 70). A low concentration of vinblastine had only a small effect on the

trans-endothelial-electrical resistance (TEER). However, when given together with PSC 833, TEER dropped to very low levels, indicating that paracellular permeability had increased considerably. This was confirmed by experiments measuring the transport of the paracellular marker fluorescein. Others have shown similar data following administration of doxorubicin and a Pgp inhibitor in an in vitro BBB model (71). In addition, in vivo drug-drug interactions at the level of Pgp can also lead to side effects, as has been suggested for the interaction between diltiazem and tacrolimus (72).

Recently, it was shown that the 5-HT_{1A} receptor antagonist flesinoxan was effluxed by Pgp in vitro as well as in vivo in rats and mdr1a(+/+) and -(−/−) mice (73). In monolayers of MDR1-transfected LLC-PK1 cells, the transport of flesinoxan could be inhibited by PSC 833, LY 335979, and verapamil. In addition, transport could be saturated at concentrations higher than 10 ug/ml, and in the in vitro BBB system, transport polarity was also observed. Following administration of 3 mg flesinoxan/kg in the tail artery of mdr1a(−/−) mice, a brain plasma ratio of 27.0 was observed, whereas in mdr1a(+/+) mice, this ratio was 12.6. In addition, the ratios in other tissues (heart, kidney, liver, lung, and spleen) were much lower in the mdr1a(−/−) as well as in the mdr1a(+/+) mice. Moreover, intracerebral microdialysis experiments were performed in rats following administration of flesinoxan. The C_{max} and the area under the curve (AUC) of the concentrations in the ECF were increased by a factor of 5–6 following co-administration of PSC 833, whereas the transport of the extracellular marker compound fluorescein to the brain was unchanged (73).

HIV infection is another disease where Pgp at the BBB limits its treatment. Besides immunological cells, HIV also affects the CNS. In 40% of AIDS patients, serious neurological disorders, such as AIDS-dementia complex, are developed, particularly at late stages of the disease (74). Furthermore, the infected brain can continuously re-infect the periphery by serving as a reservoir for the virus (75). Therefore, it is important that an anti-HIV agent passes the BBB and achieves effective concentrations in the CNS. However, due to Pgp, the concentration of these drugs in the CNS may be too low to be effective to stop HIV replication and re-infection (76). A suitable strategy to overcome these problems would be to increase the concentrations of the HIV protease inhibitors in the CNS by inhibiting Pgp at the level of the BBB. However, although it was suggested that ritonavir could be used for this purpose, it did not increase the transport of other HIV protease inhibitors in vitro or in vivo (77, 78).

The function of Pgp to limit the access of drugs to the brain, and therefore their pharmacodynamic effects in the brain, is even more clearly demonstrated for drugs that are supposed to act peripherally. The opioid receptor agonist asimadoline, which is in development as a peripherally acting analgesic, and loperamide, an antidiarrheal drug, do not enter the CNS and normally have no central effects. However, administration of these drugs to mdr1a(−/−) or mdr1a/1b(−/−) mice leads to analgesic and morphine-like effects (79). This demonstrates that Pgp in the BBB is responsible for the selective peripheral effects of those drugs in humans.

Similarly, second-generation antihistaminics were excluded from the brain by Pgp, whereas first-generation compounds were not (80).

Thus, one could say that, currently, many CNS active drugs have been shown to be Pgp substrates. Inhibition of Pgp or drug-drug interactions at the level of Pgp can therefore have serious consequences for drug therapy of CNS disorders and can even lead to CNS toxicity (81).

Modulation of Pgp-Activity

Several compounds effectively inhibit Pgp, competitively or noncompetitively. These include verapamil, R-verapamil, cyclosporin-A, PSC 833, LY 335979, GF 120918, S 9788, and RU-486 (82). Another possibility for interaction with Pgp is at its glycosylation sites. Pgp has three glycosylation sites; however, blocking of these sites with tunicamycin did not change its efflux function (83). In addition, there are various phosphorylation sites at Pgp that are phosphorylated by protein kinase A and C (84). Application of protein kinase C (PKC) inhibitors resulted in increased accumulation of Pgp substrates (85), whereas phorbol esters stimulated its phosphorylation and increased drug resistance (86). However, the problem with PKC inhibitors is that these compounds are not specific for one PKC isoenzyme. In addition, the PKC inhibitor bryostatin leads first to activation of PKC and later to a downregulation of PKC. For these reasons, PKC modulation has led to many contradicting results (87).

Recently, it was shown that oxidative stress changes Pgp expression in primary rat BCEC (brain-capillary-endothelial cells) (88). Particularly, this may have consequences for the transport of substrates in and out of the brain under disease conditions like ischemia.

Another method of Pgp regulation has been demonstrated by adrenomedullin (AM) (89). It is produced by endothelial cells in the brain and acts as a vasodilator in the cerebral circulation. It was shown that AM antisense decreased the transendothelial electrical resistance across endothelial monolayers. Treatment of these cells with AM activated Pgp, suggesting that AM acts as an autocrine mediator in the regulation of the properties of BBB endothelial cells. In addition, AM incubation decreased BBB permeability for sodium fluorescein (376 Da) but not for Evan's blue albumin (67 kDa). An interesting observation was that it also attenuated fluid-phase endocytosis.

An approach to enhance Pgp inhibition was applied by Matsuo et al. (90). They used liposomes with a covalently bound monoclonal antibody against an extracellular epitope of Pgp (MRK-16). The binding of these liposomes to K-562/ADM cells (adriamycin-resistant human myelogenous leukemia cell line) was higher than that of IgG2A-modified liposomes and liposomes without modification. In addition, when vincristin was encapsulated in all types of liposomes, it was demonstrated that the cytotoxicity of MRK-16-modified liposomes was higher than that of IgG2a and nonmodified liposomes.

There are various ways to modulate Pgp activity. Effective Pgp inhibitors have been developed; however, their therapeutic use is often limited.

MRP-EFFLUX SYSTEMS

In addition to Pgp-mediated MDR, there is also a non-Pgp-mediated MDR phenomenon. This comprises another ABC transporter subfamily that is called the MRP-family. At least seven members have been identified, and five (MRP1, -3, -4, -5, and -6) of them are expressed at the BBB [(44, 91); reviewed by Borst (92, 93)]. The MRPs are membrane-fixed systems that vary in size from 1325 to 1545 amino acids (92). They comprise two transmembrane domains of six alpha helices, a cytoplasmic linker region, and two intracellular ABCs. The linker region is essential for its transport function (94, 95). In addition, MRP1, -2, -3, and -6 have an extra domain structure comprising five additional transmembrane-segments at the animo-end (92, 96). Today, MRPs are considered amphipatic anion efflux pumps.

The MRPs transport mainly anions, but can also transport cations and neutral compounds. Two mechanisms have been proposed. One is that the anions are directly transported, and the second is that the cationic and neutral compounds are cotransported with glutathion (GSH). It has been suggested that MRPs contain dual binding sites for the direct binding of drug-GSH complexes or sequential binding of GSH. In addition, one of these binding sites may have a higher affinity for drugs and a lower affinity for GSH, whereas the second binding site is of the opposite conformation (92).

Thus, the role of MRPs at the BBB is only partially known. They play a major role in the elimination of amphipatic anions (many of them being phase II metabolites) from the endothelial compartment. This may also be their main physiological function. Based on the limited knowledge about their physiological substrates, one can only speculate about their role in disease processes at the level of the BBB.

ORGANIC ANION INFLUX- AND EFFLUX TRANSPORTERS

Due to the negative charge on the cell membrane, negatively charged compounds have difficulty entering or exiting cells. Therefore, transporters have appeared at the BBB that influx and/or efflux such compounds. Many of these transporters are called multispecific, indicating that they are able to transport several substrates. There are two main families of multispecific anion transporters: the organic anion transporter (OAT) and the organic anion transporter polypeptide (oatp) family (97). They are all membrane-fixed transporters.

Presently, eight members of the oatp-family, i.e., oatp1, oatp2, oatp3, OAT-K1, OAT-K2, OATP(A), the prostaglandin transporter (PGT), and the liver-specific transporter-1 (LST), have been identified (97). Oatp2 is localized at the apical and basolateral side of BCEC and the basolateral side of the epithelial cells of the choroid plexus (98). It transports anions like bile acids, taurocholate, cholate, estrogen conjugates, ouabain, and digoxin (99). The human OATP transporter [OATP(A)] has been identified in human BCEC and was found to transport opioid peptides [deltorphin II and (D-Pen(2),D-Pen(5))enkephalin] (100).

Presently, four OATs have been identified, comprising three subfamilies of organic anion transporters: the sodium-dependent OATs, the sodium-independent facilitators or exchangers, and active OATs that require ATP. The sodium-dependent OATs have a narrow substrate specificity and are involved in the reabsorption of anionic substances from the proximal tubules in the kidney. The active and sodium-independent OATs have a broad substrate specificity and are involved in the efflux of organic anions in the kidney and the liver (97). OAT1 is a multispecific organic anion/dicarboxylate exchanger of various organic anions [para-aminohippurate, dicarboxylates, cyclic nucleotides, prostaglandin E, beta-lactam antibiotics, nonsteroidal antiinflammatory drugs, and diuretics; (97)]. Its expression in the brain is very low. OAT3 mRNA has been identified in human and rat brain, and the transporter is involved in the efflux of (endogenous) anionic compounds (para-aminohippurate, dehydroepiandosterone sulfate) from the brain (101).

In conclusion, OATPs and sodium-independent OATs transport anionic compounds with a rather broad substrate specificity, whereas the sodium-dependent OATs have a narrow substrate specificity. Considering their substrates, it can be concluded that they have a similar physiological function as the MRPs in eliminating anionic compounds from the endothelial compartment. This may also be their major role at the BBB. Little is known about diseases related to malfunction of these transporters.

NUCLEOSIDE TRANSPORT SYSTEMS

The brain needs the influx of nucleosides because the brain is deficient in de novo nucleotide synthesis (102). Purine and pyrimidine nucleosides are necessary for the synthesis of DNA and RNA, but nucleosides also influence many other biological processes. In addition, nucleosides play an important role in the treatment of diseases, such as cardiac diseases, brain cancers, and infections [parasitic and viral; (103)]. Nucleosides are hydrophilic compounds, and the influx and efflux of these compounds is therefore mediated by a number of distinct transporters (104). Nucleoside transporters are membrane-fixed transporters and are classified by their transport mechanisms (e = equilibrative, c = concentrative), their sensitivity to the transport inhibitor nitrobenzylmercaptopurine riboside (NBMPR; s = sensitive, i = insensitive), and their substrates. Presently, there are two equilibrative transporters (ENTs: *es* and *ei*) and six concentrative nucleoside transporters [CNTs: *cif* (concentrative, NBMPR insensitive, broad specificity; N1), *cit* (concentrative, NBMPR insensitive, common permeant thymidine; N2), *cib* (concentrative, NBMPR insensitive, broad specificity; N3), *cib* (concentrative, MBMPR insensitive, broad specificity; N4), *cs* (concentrative, NBMPR sensitive; N5), and *csg* (concentrative, NBMPR sensitive, accepts guanosine as permeant; N6); (104)]. The equilibrative *es* and *ei* nucleoside transporters are widely expressed in mammalian cells and are present at cultured endothelial cells and brain capillaries (105). In these cells, the expression of concentrative transporter *cit* (N2) was demonstrated also. In other parts of the rat brain, *ei* and *es* nucleoside transport systems have

been identified, but not their localization (106, 107). Other research indicates that a sodium-dependent nucleoside transport system is present at the BBB that is not involved in the transport of nucleoside analogues (108). Recently this transporter has been cloned from a rat brain cDNA library and shown to be similar to the rat concentrative sodium nucleoside cotransporter [CNT2; (104, 109)]. In addition, very recently, the *es*-NT transporter was demonstrated at bovine BCEC by RT-PCR (8).

In conclusion, the equilibrative nucleoside transporters are particularly widely expressed in mammalians, whereas until now, the concentrative transporter (CNT2) has been identified at the BBB. Although nucleosides play a role in many biological processes and various diseases, their role at the BBB in relation to brain diseases is not clear.

LARGE NEUTRAL AMINO ACID TRANSPORTER

The large neutral amino acid transporter (LAT) is expressed at the bovine BBB. It is analogous to the L-preferring system in peripheral tissues and a membrane-fixed transport system. Large amino acids are transported via this system, comprising a heterodimer of the 4F2hc heavy chain and the LAT1 light chain, which is similar to other amino acid transporters (110). From cloning experiments and full-length cDNA, it can be derived that the bovine LAT1 comprises 505 amino acids and has a predictive molecular mass of 55 kDa (111). Using Northern blotting experiments, it was estimated that LAT1 was profoundly upregulated in brain capillaries. The amount of LAT1 mRNA in bovine BCEC was very high compared to other tissues, such as lung, spleen, testes, and heart (111). However, the K_m of the L-system at the BBB was much smaller (10–100 μM) than of those in peripheral tissues [1–10 mM; (112, 113)]. In addition, the K_m of LAT1 at the BBB is similar to the plasma concentration of circulating large amino acids, which means that this transporter is saturated under normal conditions. This makes the brain vulnerable to pathological effects of hyperaminoacidmias (114).

cDNAs of rat and human LAT2 have also been cloned, and it was suggested that LAT2 was expressed at the BBB (115). The K_m of the LAT2 transporter for leucine was shown to be 120 ± 34 μM (115) and approximately 10 times greater than the K_m of leucine by the LAT1 transporter (112). Recently, it was shown that LAT1 is the predominant functional active LAT isoform at the microvascular endothelium of rat brain (116).

The LAT system has been used for the transport of various compounds to the brain. Variations in the cerebellum to plasma ratio at late times in 6-[18F]fluoro-L-DOPA studies are consistent with competitive binding of large neutral amino acids (LNAAs) for the LAT at the BBB (117). In addition, it was shown that oral administration of phenylalanine inhibited the uptake of an artificial amino acid [(11C)-aminocyclohexanecarboxylate] in human brain (118). Melphalan, a nitrogen mustard derivative of the neutral amino acid L-phenylalanine, was transported to the brain via the LAT system at the rat BBB. In addition, it was shown that melphalan competed with phenylalanine for the LAT system (119).

Other data show that under disease state, transport systems may be less available to transport compounds into the brain. It was demonstrated that the transport of phenylalanine by LAT into the brain of patients with phenylketonuria was blocked (120). In addition, EEG analysis revealed that brain activity was acutely disturbed when phenylalanine was given orally without other LNAAs. Following administration with LNAAs, phenylalanine influx was completely blocked and no influence on EEG could be observed.

On the other hand, it was demonstrated that 7-chlorokynurenic acid and 5,7-dichlorokynurenic acid were efficiently taken up by the LAT in the brain when they were administered as their amino acid precursors L-4-chlorokynurenine and L-4,6-dichlorokynurenine (121). In the brain, these precursors were converted again to the parent compounds.

It can be concluded that the LAT1 and -2 transporters are present at the BBB. The LAT1 seems to be more expressed than the LAT2, whereas its K_m is approximately a factor of 10 less. The applicability of this transporter for targeted drug transport to the brain has been demonstrated. In addition, its role at the BBB in diseases has been demonstrated with phenylketonuria where the transport of phenylalanine into the brain was blocked.

ROLE OF TRANSPORT PROCESSES AT THE BLOOD-BRAIN BARRIER: RECEPTOR-MEDIATED TRANSPORT OF DRUGS

Transferrin-Receptor-1 and -2

The brain needs iron-III (Fe) for processes like storage and transport of oxygen, electron transport, DNA synthesis, oxidation-reduction reactions, and cell division (122). Fe-containing Tf (holo-Tf) is transported into the cell by the transferrin-receptor (Tf-R). The Tf-R is heterogeneously distributed within the brain. It is a homodimer of two identical transmembrane subunits, each of 90–95 kDa (123). The receptor subunits are linked by two disulfide bonds (124) and each receptor subunit consists of three domains: a large extracellular C terminus consisting of 671 amino acids, a 28-residue intramembrane part, and an intracellular N-terminal domain of 61 residues (123). The extracellular part of the receptor contains a trypsin-sensitive site and cleavage leads to loss of Tf-binding activity. The Tf-binding site is located at the extracellular domain of the receptor, and each receptor subunit binds one Tf molecule. Tf-R has been identified in capillary endothelial cells in the brain (125).

A second Tf-R (Tf-R2) has been identified that shares 45% identity and 66% similarity in its extracellular domain with Tf-R (126). However, there are currently no indications that the Tf-R2 is expressed at BCEC.

The internalization of Tf occurs via an endocytotic clathrin-coated process, and the internalization signal YTRF is also recognized by clathrin lattices in the trans-Golgi (127). In vitro studies have indicated that there are two different endocytic pathways of clathrin-coated vesicles in cells: a short-term (10–20 min) recycling

pathway and a long-term (2–3 h) recycling pathway involving the movement of Tf-R from endosomes to the Golgi complex (128).

The expression of Tf-R in brain cells is dependent on the developmental stage and it varies with region, cell type, and age (129). In addition, it was shown that Tf-R expression in endothelial cells increased under conditions of Fe deficiency (130), and that oxidative stress leads to a rapid alteration of Tf-R trafficking and a downregulation in K562 and HL60 cells (131).

Recently, several genes have been discovered that encode for proteins that regulate transmembrane iron transport. These are the HFE gene (132), associated with hereditary hemochromatosis, and the DMT1/Nramp2 gene, which encodes for a divalent metal transporter (133). The HFE-protein binds Tf-R tightly at the pH of the cell surface and negatively regulates Tf-mediated iron uptake in transfected cells (134). DMT1 is an iron transporter that is also essential for the transport of iron from the endosomal membrane to the cytosol. In addition, it seems that the regulation of iron homeostasis in the brain is different or more complicated than in other tissues, and that other proteins, like the lactoferrin receptor, melanotransferrin, ceruloplasmin, and the DMT1-transporter, play an important role (135).

It is known that only part of the Tf-Rs are available at the cell surface. At porcine BCEC (129) and bovine BCEC (136), approximately 10% Tf-R was found at the surface of the cells. In our laboratory, we have found a similar percentage at the surface of bovine BCEC, whereas others found that extracellular expression was lower by a factor of three (138).

Internalization via the Tf-R has been demonstrated for various compounds, including systems comprising a compound that has been coupled via a spacer to rat Tf-R monoclonal antibodies [MAb-Tf-R; (139)]. In addition, pegylation of such systems prolonged the circulation time in plasma and resulted in increased brain uptake of brain-derived neurotrophic factor (BDNF). With these systems, it was possible to get neuroprotection against transient focal brain ischemia (139). Further, liposomal drug reservoirs coupled to MAb-Tf-R have been used to deliver plasmid DNA encoding beta-galactosidase (140) or the Photimus pyralis luciferase gene (pGL2 plasmid) to the brain (141). Following intravenous administration in pegylated liposomes without Ab or with the OX-26 MAb-Tf-R, the ^{32}pGL2 plasmid was preferentially taken up by liver and brain, and to a lower extent, by kidney and heart.

Another iron-transporting protein is the melanotransferrin (MTf), or the P97 protein. Its expression has been demonstrated in human brain capillary endothelial cells and it is bound to the plasma membrane via a glycosylpyosphatidylinositol (GPE) anchor. It transports iron independently from the Tf-R route. A role for MTf has been suggested in Alzheimer's disease because its expression has been shown in reactive microglia from amyloid plaques (142).

In conclusion, Tf-Rs are interesting systems. They can internalize relatively large substances or particulate systems, and are therefore suitable for drug targeting. However, Tf-Rs are also present in the liver and in bone marrow, which limits their selectivity and applicability for drug targeting to the brain. The expression of Tf-R is strongly regulated and influenced by many factors.

SCAVENGER RECEPTORS

The presence of the scavenger receptor was demonstrated in bovine and porcine BCECs (143, 144). It is an internalizing membrane-located multifunctional receptor that can transport ligands to the lysosomes where they can be degraded. There are at least nine distinct scavenger receptors that have been cloned (145). These have been organized into classes (A, B, D, etc.). Further subclassification has been done into various types. Particularly, the SR-BI receptor is expressed at BCEC. SR-BI is a member of the CD36 superfamily of proteins. It comprises 509 amino acids, and the rodent SR-BI is identical to the human SR-BI (146). The glycoprotein is heavily N-glycosylated and palmitoylated at the cysteins in the C-terminal cytoplasmic and transmembrane domains. It has a large extracellular loop that is anchored to the plasma membrane and short extension in the cytoplasm. The receptor clusters in caveolae cholesterol-rich lipid domains (147) and there is evidence that SR-BI undergoes rapid endocytosis from the plasma membrane of primary mouse hepatocytes and CHO cells expressing SR-BI (148). The receptor recognizes a broad variety of substrates, particularly chemically modified lipoproteins like acetoacetyl low-density lipoprotein, oxidized LDL, malondialdehyde-conjugates of either LDL or BSA, some polyanions, apoptotic cells, unmodified LDL, and VLDL (145). In addition, it was shown that this receptor binds HDL with high affinity and removes cholesteryl esters from the HDL particle, which is resecreted out of the cell (149). Further, it was shown that acetylated-LDL (AcLDL) was saturably taken up by BCEC, and that the rate of degradation of AcLDL was 20-fold lower in BCEC than in peripheral endothelial cells. In addition, association and degradation could not be influenced by 100 μM chloroquine or 10 mM ammonium chloride, indicating that the lysosomal pathway was at least a minor intracellular route following internalization (143).

The binding of other negatively charged compounds by the scavenger receptor at bovine BCECs was demonstrated by Nakamura et al. (150). They found that binding of naked plasmid DNA was saturable at 4°C and was inhibited by polyinosinic acid and dextran sulphate, which are typical ligands for the macrophage scavenger receptor. In addition, polycytidylic acid or EDTA could not inhibit binding.

Recently, it was shown that compounds that are bound to HDL have the possibility to be internalized by a "piggy-back"-like mechanism. HDL-associated alpha-tocopherol was selectively taken up by SR-BI by porcine BCECs (144). It was shown that the alpha-tocopherol uptake exceeded the uptake of HDL(3) particles up to 13-fold, suggesting a selective uptake of this compound without the concomitant internalization of the lipoprotein particle.

Apolipoprotein (apo) A-I expression was demonstrated in porcine brain capillaries, suggesting an independent lipid metabolism in the brain (151). Apo A-I is the major protein component of HDLs, which are responsible for reverse cholesterol transport from various tissues to the liver via the SR-BI receptor. Further research indicated that apo A-I was effluxed by porcine BCEC, whereas aortic endothelial cells did not. In addition, apo A-I–inducing compounds, such as cholesterol,

insulin, and retinoic acid, could upregulate apo A-I in these cells. These data indicate that at the porcine BCEC apo A-1 is effluxed apparently by the SR-BI receptor.

Other research demonstrated the uptake of the soluble beta-amyloid peptide sAß, which is the major part of Alzheimer's neuritic plaques. sAß was shown to be complexed to ApoJ in HDL(3)- and VHDL (very-high-density-lipid)-particles, and it was suggested that these particles were involved in the delivery of sAß across the BBB (152). Makic et al. (153) found binding of the soluble monomeric 1–40 amino acid peptide Alzheimer amyloid-beta (sA-beta$_{1\text{-}40}$) at the class A, type I scavenger receptor (SB-AI) and the receptor for advanced glycosylation products (RAGE) at human BCECs. Binding was polarized and could be inhibited by anti-RAGE antibodies and acetylated low-density lipoprotein for 63% and 33%, respectively. Transfected CHO-cells overexpressing the SR-AI or RAGE receptor internalized sA-beta$_{1\text{-}40}$, which remained intact. In addition, transcytosis occurred for more than 94%. It was temperature and time dependent and could be partially blocked by anti-RAGE antibodies (36%), but not by 100-fold excess of cold (sA-beta$_{1\text{-}40}$). These observations indicate the importance of the SR-AI receptor at the BBB. It functions as a multiligand receptor, and in that respect, looks similar to the MDR1-, the various MRP transporters, and the various cytochromes P450 (144, 154). However, scavenger receptors also bind particles and pathogens. The latter has lead to the suggestion that these receptors participate in the innate immune system by serving as pattern recognition receptors (155) that bind to a variety of components of pathogens (156).

In conclusion, scavenger receptors are multifunctional receptors with a wide substrate specificity. Particularly, the SR-AI and SR-BI are expressed at BCECs. In addition, these receptors are widely expressed in mammalian tissues, particularly in liver, macrophages, endothelial cells, etc. This makes these receptors less suitable for targeting drugs to the brain. Their role at the BBB seems to be a very important one because the SR-AI receptor seems to be involved with neurodegenerative diseases. In addition, the SR-BI receptor has been shown to play a role in the transport of cholesteryl esters at the BBB. Therefore, malfunction of this receptor can also result in atherosclerotic events leading to neurodegenerative processes in the brain.

CONCLUSIONS

It can be concluded that the role of drug transporters at the BBB can be a critical one. Next to the transport of nutrients like amino acids, glucose, and nucleotides, other systems are present to influx or efflux substances that otherwise cannot be eliminated from the BBB endothelial compartment. Particularly, charged substances will accumulate in the cell when efflux transporters are inhibited or less functional. This may result in toxic concentrations of these substances that can ultimately influence BBB functionality and integrity. Such a situation can occur under acute inflammatory conditions but also with diseases like multiple sclerosis and Alzheimer's disease. Under such conditions, CNS homeostasis can be disturbed, resulting in reduced (cognitive) performance and behavior. It is therefore

very important to understand the role of drug transporters at the BBB and to interfere with their functionality under disease or pathophysiological conditions to restore CNS homeostasis.

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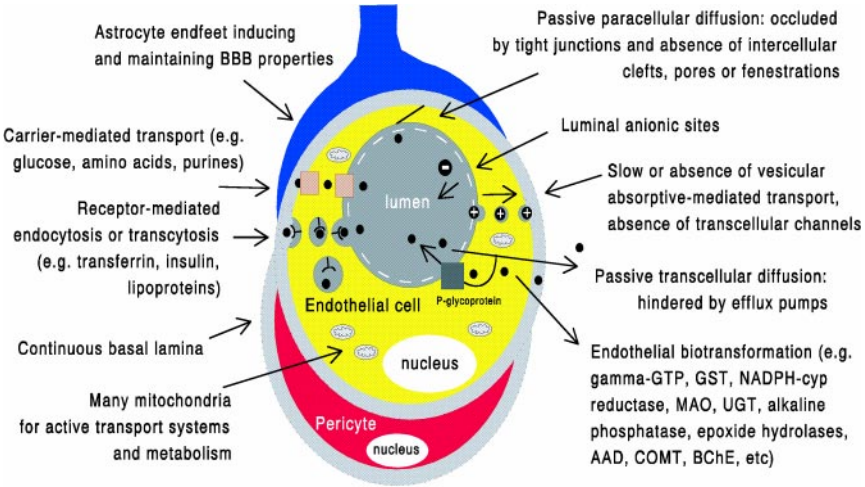

Figure 2 Schematic illustration of the (transport) properties of the blood-brain barrier. Shown is the influence of astrocyte endfeet at the brain capillary endothelial cell. This cell has narrow tight junctions, low pinocytotic activity, many mitochondria, and luminal anionic sites that hinder the transport of negatively charged compounds. Passive hydrophilic transport occurs via paracellular diffusion (tight junctions), whereas passive lipophilic transport is a transcytotic process. Adsorptive-, receptor-, and carrier-mediated transport has been indicated. The metabolic properties of the BBB are illustrated by the various enzymes at the BBB [from (157), with permission].