Small Molecular Drug Transfer across the Blood-Brain Barrier via Carrier-Mediated Transport Systems

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Summary: Because of the physiological nature of the blood-brain barrier (BBB), transport of chemical compounds between blood and brain has been widely believed to occur by means of passive diffusion, depending upon the lipophilicity of the compounds. However, discrepancies exist between the lipophilicity and apparent BBB permeation properties in many cases, and these discrepancies can be ascribed to the existence of multiple mechanisms of drug transport through the BBB. Molecular identification and functional analysis of influx transport proteins (from blood to brain) and efflux transport proteins (from brain to blood) have progressed rapidly. Therefore, the BBB is now considered to be a dynamic interface that controls the influx and efflux of a wide variety of substances, including endogenous nutrients and exogenous compounds such as drugs, to maintain a favorable environment for the CNS. This review focuses on the role of transport systems in the uptake of xenobiotics, including organic anionic/cationic and neutral drugs, across the BBB into the brain, as well as on strategies to increase drug delivery into the brain by blocking efflux transport protein function, or to reduce CNS side effects by modulating BBB transport processes. **Key Words:** Transporter, blood-brain barrier, influx transport, organic anions, organic cations, drug delivery.

INTRODUCTION

Transporters that mediate influx and efflux of endogenous nutrients and exogenous toxins including drugs across biological membranes are necessary for the survival of mammalian species. These transporters are classified into the solute carrier superfamily, for which gene symbols are prefixed with *SLC*, and ATP binding cassette transporters, for which gene symbols are prefixed with *ABC* (http://www.gene.ucl.ac.uk/nomenclature/genefamily.shtml).

The CNS contains cellular barriers that maintain homeostasis by elimination of toxic metabolites generated in the brain. The barriers that limit the concentrations of toxins and xenobiotics in the interstitial fluids of the CNS are the blood-brain barrier (BBB), consisting of brain capillary endothelial cells, and the blood-CSF barrier, consisting of epithelial cells. Both barriers have cellular tight junctions and express transport systems that serve to actively transport physiologically important nutrients such as glucose, amino acids, etc. into the brain, and to actively import or export various xenobiotics including drugs and their metabolites. These transport systems are increasingly being recognized as important determinants of drug distribution into and efflux from the brain.

The present review is focused on influx, but not efflux, transporters that have been identified at the molecular level and have relevance to drug delivery to the brain, especially the SCL transporters and related transport systems expressed in the BBB.

Carrier-mediated transport of amino acid-like drugs

Essential amino acids need to be transferred from the bloodstream to the brain, and the presence of specific transporters has been documented.[1] Transporters for amino acids have in general been classified in terms of their functional characteristics such as sodium dependence and substrate specificity. Large neutral amino acids such as phenylalanine, tyrosine, and leucine are transported by System L across the BBB in a sodium-ion-independent manner, and this system is characterized by specific reduction of its activity by 2-aminobicyclo2,2,1-heptane-2-carboxylic acid (BCH).[1] This system is supposed to be important for drug delivery because System L has broad substrate

specificity for relatively large molecules. System L is presumed to transport CNS-acting drugs such as levodopa, baclofen, and gabapentin across the BBB.[1,2] The *in vivo* significance of System L in levodopa transport across the BBB was indirectly shown by the decreased effectiveness of levodopa after a protein-rich meal in humans.[3] The decrease in pharmacological activity was explained in terms of lowered distribution of levodopa into the brain due to saturation of System L with high plasma concentrations of amino acids generated from the meal.[3] Recently, one of the System L transporter genes has been cloned and named LAT-1 (*SLC7A5*).[4,5] LAT-1 is characterized by sodium-ion independence with Michaelis constant (Km) values varying from 10 μM order for leucine to 1 mM order for glutamine, depending on the hydrophobicity and side-chain conformation of the amino acids.[6] LAT-1 is highly expressed at the BBB and requires coexpression with cell surface glycoprotein 4F2 heavy chain to be adequately expressed on the cell membrane surface.[4–7]

LAT-2 (*SLC7A8*), which shows differential substrate specificity from LAT-1, may also function as a transporter for amino acids and drugs at the BBB.[8–10]

Hexose transport systems

Glucose is essential for brain function and glucose crosses the BBB via a specific transporter. Because the capacity of the glucose transporter at the BBB is significantly higher (1420 nmol/min · g tissue) than those of other nutrient transporters (91 nmol/min · g tissue for monocarboxylic acid, 28 for neutral amino acid and 11.3 for amine transport systems), it is expected to be applicable for the brain delivery of drugs.[11] There are two types of glucose transporters, namely sodium-dependent (secondary active) and -independent (facilitative) transporters that are molecularly classified into the SGLT (*SLC5A*) and GLUT (*SLC2A*) families, respectively. Sodium-independent glucose transporters are thought to be functional in the brain, although the possible involvement of members of the SGLT transporter family cannot be completely excluded.[12] Sodium-independent GLUT1 (*SLC2A1*) transporter is present at brain capillaries, as well as choroid plexus and neuronal cell membranes. Interestingly, the glycosylation state of GLUT1 is variable among regions, resulting in different molecular masses of 54, 47, and 42 kDa in brain capillaries, choroid plexus and neuronal cells, respectively.[13] Although further molecular studies on glucose transport at the BBB are needed, the glucose transporter is likely to be useful for delivery of membrane-impermeable compounds, and several authors have found that sugar analogues may cross the barrier via this transporter. Modification of D-glucose with an *O*-methylsulfonyl moiety was tried for delivery of the alkylating agent busulfan into the brain.[14] Furthermore, glycosylation of the opioid peptide dermor-

phin was tried.[15] In the case of peptide compounds, glycosylation may stabilize the peptides, rather than enhance the BBB permeability. More studies are needed on the utilization of the glucose transporter to obtain enhanced permeability across the BBB.

Monocarboxylate transport family

Lactic acid and other monocarboxylates are important metabolic products and energy substrates of brain metabolism in mammalians, and their movement across the BBB is regulated by specific uptake and efflux transporters. The carrier-mediated transport system for these drugs is presumably common to short-chain fatty acids such as acetate.[16,17]

Earlier *in vivo* studies suggested the presence of a monocarboxylate transport (MCT) system at the BBB as demonstrated by stereospecific and saturable transport of lactate and pyruvate across the BBB.[18–20] Other *in vivo* studies in our laboratories indicated that several compounds including acetate, propionate, butyrate, benzoic acid, salicylic acid, nicotinic acid, and some β-lactam antibiotics may be transported by the MCT at the BBB.[21] Numerous studies using primary cultured brain capillary endothelial cells as an *in vitro* BBB model support these *in vivo* results.[22,23]

Currently, at least eight members of the MCT (*SLC16A*) family have been discovered in various tissues and orthologs to each have been identified in a variety of species.[24] Each isoform possesses unique biochemical properties, such as kinetic constants and sensitivity to MCT inhibitors. Post-translational regulation of MCT1 (*SCL16A1*) and MCT4 (*SLC16A3*) occurs, in part, by interaction with CD147, an accessory protein that is necessary for trafficking, localization, and functional expression of these transporters.[24]

Among MCT family members, MCT1 is expressed in the epithelium of the small intestine and colon, the BBB and other tissues, including muscle. MCT1 expressed at the BBB is likely to provide a mechanism for the blood-to-brain and brain-to-blood transfers of carboxylated pharmaceutical agents and other exogenous monocarboxylates. Rat MCT1 stably expressed in cultured MDA-MB231 cells exhibited pH-dependent, stereospecific transport of lactic acid and R- and S-mandelic acid,[25] as observed previously in the transport of pyruvic acid by MCT1 in Chinese hamster ovary (CHO) cells.[26] Interestingly, benzoic acid, which had long been believed to cross membranes by passive diffusion according to the pH partition hypothesis, is a good substrate[25] for rat MCT1. Transport of benzoic acid across the BBB studied by using primary cultured cells of rat brain capillary endothelial cells (RBEC1) and by the *in vivo* Brain Uptake Index (BUI) method exhibited Km values comparable with that in MCT1-transfected cells. In addition,
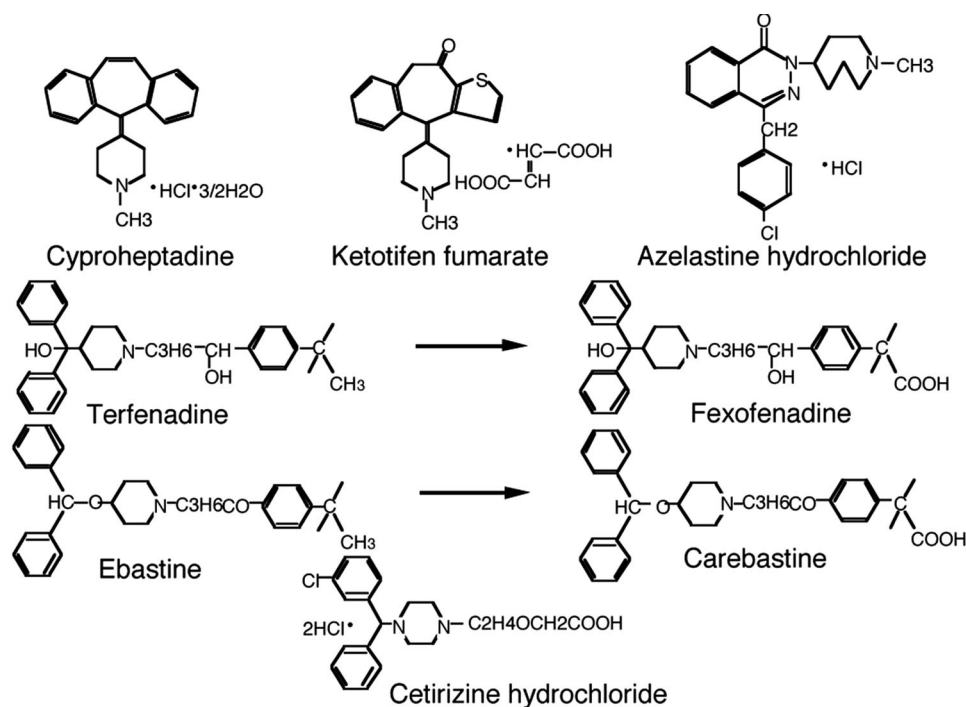
FIG. 1. Chemical structures of H1 antagonists.

pH dependence, substrate specificity, and stereospecificity were comparable in all four experimental systems. Furthermore, when expression of MCT1 in RBEC1 cells was depressed by transfection of an antisense oligonucleotide against rat MCT1, the uptake of benzoic acid was abrogated.[17] These observations demonstrate that MCT1 is responsible for monocarboxylate transport at the BBB. MCT1 is likely to transport relatively small molecules having a monocarboxylate moiety and with a molecular weight of up to ~200.

Immunoblotting. immunohistochemical, and electron micrographic techniques using MCT1-specific antibodies have shown that MCT1 is expressed on the luminal and abluminal membranes of brain capillary endothelial cells, and the expression seen in sucking rat brain is much stronger than that in adults. MCT1 may be responsible for the transport of some organic anions from brain to endothelial cells and/or from endothelial cells to blood, because it is expressed on both the luminal and abluminal membranes of brain capillary endothelial cells, and it seems to operate bidirectionally.[16,24,25] It is also possible that lactic acid and other monocarboxylates are excluded from the brain via the abluminal MCT1 to maintain homeostasis.[24] Microdialysis studies of probenecid in the rat brain interstitial fluid suggest that the restricted distribution of probenecid[27] and 6-mercaptopurine[28] in the brain is the result in part of efflux via MCT1, as well as a contribution by MRP2 and organic anion transporters that are known to be expressed at the BBB.[29]

There are several studies showing that an anticonvul-sant, valproic acid, is efficiently transported across the BBB.[30,31] When uptake of valproic acid was measured by an *in situ* brain perfusion method, it was saturable, with Km values between 10 and 20 mM, depending on the measured brain regions. Uptake of valproic acid was reduced in the presence of medium-chain fatty acids such as hexanoate, octanoate, and decanoate, but not propionate or butyrate, indicating that valproic acid is taken up into the brain via a transport system for medium-chain fatty acids, not short-chain fatty acids. Interestingly, acetic acid, probenecid, and dicarboxylic acids, such as succinate and glutarate, increased the brain uptake of valproic acid.[27] These observations may be ascribed to participation of an anion exchange mechanism similar to that reported for renal transport of organic anions such as p-aminohippuric acid,[32] or to inhibition of efflux of valproic acid from brain to blood, or to some other indirect effect. Based on these reports, valproic acid is thought to be transported bidirectionally between blood and brain across the BBB via two distinct mechanisms, monocarboxylic acid-sensitive and medium-chain fatty acid-sensitive transporters, for efflux and uptake, respectively.

Organic anion transporter family and organic anion transporting polypeptide transporter family

Four mammalian organic anion transporter (OAT) isoforms (OAT1, OAT2, OAT3 and OAT4) have been identified.[33,34] OAT1 (*SLC22A6*) and/or OAT3 (*SLC22A8*) are likely to play the important roles in the transport of several organic anions in the brain. However, the localization of OATs in the brain is not clear. P-Aminohip-

puric acid is a substrate of both rOAT1 and rOAT3 and is actively transported from brain to plasma across the BBB and so OATs are outside the scope of this review. Members of the multispecific organic anion transporting polypeptide superfamily, rOatp1 (*Slc21a1*) and rOatp2 (*Slc21a4*) in rats and hOATP-A (*SLC21A3*) in human, have been reported to be expressed at the BBB.[35,36] rOatp2 is expressed on both the luminal and abluminal membranes of brain capillary endothelial cells.[37] In functional expression studies in *Xenopus laevis* oocytes, rOatp2 mediated the uptake of bile acids such as taurocholate (Km = 35 μM) and cholate (Km = 46 μM), estrogen conjugates such as 17β-estradiol-glucuronide (Km = 0.24 μM), and cardiac glycosides such as ouabain (Km = 470 μM) and digoxin (Km = 0.24 μM).[37] Although most of the compounds indicated above are common substrates of several rOatp-related transporters, high-affinity uptake of digoxin is a unique feature of rOatp2. It is well known that digoxin overdose induces several types of central nervous side effects. These results indicate that oatp2, which is highly expressed in brain, as well as liver and kidney, may play an especially important role in the brain accumulation and toxicity of digoxin and in the hepatobiliary and renal excretion of cardiac glycosides from the body.[36] Recently, OATP-B (*SLC21A9*), which transports estrone-3-sulfate,[38,39] has been reported to be expressed in brain capillary endothelial cells,[40] but not in the whole brain.[38] OATP-B is also expressed in the liver and intestine. In the intestine, OATP-B expressed at the brush-border membrane of epithelial cells works effectively at the acidic microclimate pH (around 6), rather than at pH 7, in the absorption direction for various compounds including taurocholate, estrone-3-sulfate, dehydroepiandrosterone-sulfate, pravastatin, and hexofenadine,[41,42] Although the role in drug transport of OATP-B expressed at the BBB is unclear, lipophilic 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors such as the acid forms of simvastatin and lovastatin, which cause sleep disturbance as a CNS side effect, are likely to be transported via OATP-B even under a neutral pH condition. This speculation is based on previous observations from *in vitro* and *in vivo* BBB transport studies in our laboratory that the acid forms of simvastatin and lovastatin were taken up significantly via organic anion sensitive transport systems, but the BBB transport of pravastatin (having no significant CNS side effect) is negligible.[43,44]

Organic cation transporter (OCT and OCTN) family

Members of the organic cation transporter (OCT, *SLC22A*) family have been molecularly identified in the rat, rabbit, and human.[45] They transport a variety of cationic compounds, including monoamine neurotransmitters, classical organic cation transporter substrates such as tetraethylammonium (TEA) and choline, neurotoxic 1-methyl-4-phenylpyridinium (MPP), and other cationic compounds. Rat OCT3 (*SLC22A3*) and human OCT2 (*SLC22A2*) are expressed in the brain, but it is thought that they participate in the regulation of neurotransmitters in neurons rather than at the BBB.[46,47]

We have isolated a new family of organic cation/carnitine transporters, OCTNs (OCTN1-3) from human and mouse.[48–50] OCTN2 (*SLC22A5*) was also cloned from rat intestine.[51] The amino acid sequences of OCTN1 (*SLC22A4*), OCTN2 and OCTN3 show low but significant similarity to those of OCT family members and OCTNs were initially thought to be organic cation transporters. However, subsequent studies clarified that their physiological function was as sodium ion-dependent transporters for carnitine, which is important in the metabolism of fatty acids.[52] Mutation of OCTN1 and OCTN2 have been reported to cause Crohn's disease[53] and primary carnitine deficiency,[52] respectively.

OCTN1 and OCTN2 transport organic cations such as TEA in a pH-dependent or an Na$^+$-independent manner.[54–57] On the other hand, OCTN2 mediates carnitine transport in a Na$^+$-dependent manner.[49,50,56] OCTN2 mRNA is strongly expressed in human kidney, placenta, heart, and skeletal muscle, and weakly expressed in brain, lung, and liver.[49,50,56] OCTN2 behaves as an exchange transporter in Na$^+$-dependent carnitine reabsorption in the kidney and mediates Na$^+$-independent secretion of organic cations.[58] OCTN2 was confirmed to be expressed in primary cultured and freshly isolated brain capillary endothelial cells of mice, rats and humans.[59,60] Freshly isolated brain capillary endothelial cells from Octn2 gene-deficient mice (jvs), when compared with those from normal mice exhibited a significant reduction of Na$^+$-dependent carnitine transport. The brain-to-plasma concentration ratio (Kp) of carnitine after intravenous administration was demonstrated to be significantly decreased in jvs mice compared with that in normal mice.[60] A very similar decrease of the Kp value of brain in jvs mice was observed for TEA,[57,58] indicating that OCTN2 plays a significant role in BBB transport of some organic cations, as well as carnitine.

Although the significance of the organic-cation transport function of OCTN2 remains unknown, many organic cations recognized by OCTN2 as substrates are pharmacologically active and are currently used as therapeutic agents.[58] By using jvs mice, we showed that mOCTN2 can work as an efflux transporter for intracellular TEA when extracellular carnitine is taken up into tubular cells in a Na$^+$-dependent manner.[58] Therefore, OCTN2 may play a significant role in the cellular uptake and/or efflux of therapeutically important organic cations in the body.
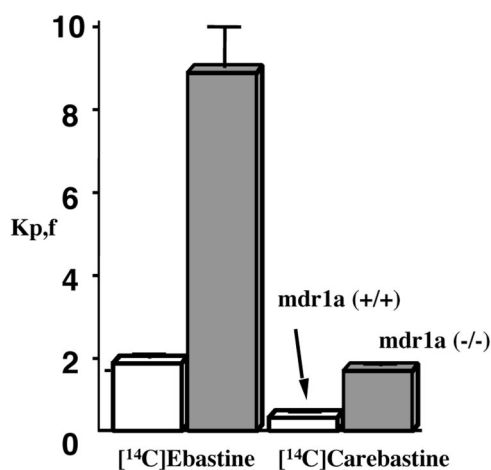
FIG. 2. Brain-to-plasma free concentration ratios of [^{14}C]ebastine and [^{14}C]carebastine 6 min after intravenous administration to wild-type mice [mdr1a(+/+)] and to mdr1a gene knockout mice [mdr1a(−/−)]. Data mean ± SEM of four to seven rats.

Utilization of BBB carrier-mediated transport systems to prevent CNS side effects of nonsedative H$_1$ antagonists

There are many cationic drugs that are effective or toxic in the CNS. At present, the molecular characteristics of the transporters for cationic drugs are not fully established, so the results of membrane physiological studies on cationic drug transport at the BBB are summarized in this review. Transport mechanisms across the BBB may include carrier mediated processes using some types of organic cation transporters including OCTNs in parallel with passive diffusion for lipophilic cationic drugs. Among H$_1$ antagonists, there are several derivatives that exhibit a sedative side effect, whereas such an effect by pravastatin is negligible. Differential distribution into the brain may explain the differences of CNS effects.

In previous studies, we found that mepyramine was transported into the brain via a carrier-mediated mechanism.[61,62] A classical H$_1$ antagonist, mepyramine is taken up by brain capillary endothelial cells via a saturable mechanism that is inhibitable by chlorpheniramine, diphenhydramine, and other cationic compounds, whereas choline, hemicholinium-3, or anionic drugs showed no effect. Other H$_1$ antagonists such as cyproheptadine, ketotifen, emedastine, and azelastine (see the chemical structures in FIG. 1) are also likely to be taken up by a common mechanism, based on the observed mutual inhibitory effects. However, the inhibitory potency of cetiridine was the lowest among them.[63] Because cetiridine has a carboxylic acid moiety within the molecule, it is a zwitterionic derivative. Accordingly, H$_1$ antagonists, which carry only cationic charges, have higher affinity for the responsible transporter and zwitterionic derivatives seem to have lower affinity, resulting in less effect on the CNS.[63] To avoid the sedative effect of H$_1$ antagonists, it may therefore be advantageous for

them to carry anionic charge. The transporter responsible for mepyramine uptake showed lower affinity for zwitterionic H$_1$ antagonists such as cetirizine than for cationic derivatives such as ketotifen and cyproheptadine. Accordingly, we postulated that zwitterionic H$_1$ antagonists could exhibit lower distribution to the brain.[61–63]

Recently, Cvetkovic et al.[64] studied the cellular uptake and efflux mechanisms of a nonsedating antihistamine, fexofenadine, which is a metabolite of cationic and sedating terfenadine, and identified organic anion-transporting polypeptides (oatp) and P-glycoprotein as uptake and efflux transporters, respectively. Because tefenadine and ebastine are structurally similar to the classical H$_1$ antagonists as shown in Figure 1, they may be transported into the brain via the same system as that suggested for mepyramine uptake. However, ebastine does not exhibit sedative properties in a practical antihistaminic dose-range.[65] Ebastine is rapidly metabolized to an active zwitterionic metabolite, carebastine, in both experimental animals and human.[65] Carebastine also has a high and selective H$_1$ receptor antagonistic activity[66] and is considered to be a major contributor to the activity of the parent drug. The intestinal absorption, distribution, metabolism, and excretion of ebastine and carebastine have been studied in rats.[63] Carebastine showed a limited distribution to the brain.[67]

The steady-state uptake of [^{14}C]carebastine by P-glycoprotein-overexpressing K562/ADM cells was significantly lower than that by the parental drug-sensitive cell line K562, and that by primary cultured bovine brain capillary endothelial cells (bovine BCECs) was increased in the presence of nonlabeled carebastine and verapamil. The initial uptake of [^{3}H]mepyramine by bovine BCECs and RBEC1 was strongly inhibited by ebastine, whereas zwitterionic carebastine was slightly inhibitory. As shown in Figure 2, the values of brain-to-plasma unbound concentration ratio (Kp,f) in mdr1a(−/−) mice were increased 5.8-fold and 9.0-fold for [^{14}C]ebastine and for [^{14}C]carebastine, respectively, compared with those in mdr1a(+/+) mice.[68] From these observations, carebastine was shown to be a substrate for P-glycoprotein-mediated efflux from the brain at the BBB. The relatively low affinity of carebastine for the mepyramine transport system also limits the brain distribution of carebastine after an oral dose of ebastine.[64]

Other drugs

Clonidine, an activator of α-2 adrenergic receptor, was reported to be taken up by cultured porcine brain capillary endothelial cells via a carrier-mediated mechanism with a Km of 1.34 mM, in a pH-dependent manner.[69] However, permeation of clonidine across the BBB is not high regardless of the participation of a transporter, perhaps due to trapping in the cells and/or the involvement of an efflux transporter other than P-gp.

Fentanyl, a μ-opiate agonist, is effective in the CNS. Its transport across the BBB may be mediated by a transporter with an apparent Km of 3.2 μM and is inhibitable by verapamil, although the functional characteristics have not been clarified yet.[70] Interestingly, although fentanyl may be effluxed by P-gp, its brain distribution is high. High BBB distribution in spite of the participation of an efflux transporter may be explained by higher efficiency of the influx transporter than that of the efflux one. So, it is possible that multiple influx and efflux transporters are involved in fentanyl transport at the BBB,[70] as observed for H$_1$ antagonists. The *in vivo* uptake of pentazocine, a narcotic-antagonist analgesic, by rat brain was significantly inhibited by lidocaine, imipramine and propranolol, as well as by H1 antagonists such as mepyramine and diphenhydramine.[71] Pentazocine, in spite of efflux transport by P-glycoprotein at the BBB, is also highly distributed into brain by a carrier-mediated transport system that may widely recognize cationic drugs.[72]

Peptides

Some small-peptide transport systems other than PepT1, PepT2, and HPT expressed in the intestinal and renal epithelial cells have been suggested to exist at the BBB.[73,74]

Enkephalins were demonstrated to be taken up efficiently into the brain, and the involvement of a saturable mechanism for the transport of leucine enkephalin was shown by the *in situ* vascular brain perfusion technique.[73,74] Several other peptides, thyrotropin-releasing hormone,[75] arginine-vasopressin,[76] Peptide-T,[77] α-melanocyte-stimulating hormone[78] and δ sleep-inducing peptide,[79] have been shown to cross the BBB and some of them showed saturable uptake, suggesting participation of carrier-mediated transport. The peptide transport mechanisms involved are classified as peptide transport systems PTS-1 to -5,[73,74] although these transporter proteins have not been isolated as yet. Recent findings[80] indicated that OATP-A (SLC21A3) plays an important role in carrier-mediated transport of opioid peptides, such as [D-penicillamine-2,5]enkephalin and deltorphin II, across the BBB.

Brain uptake of glutathione, a tripeptide, measured by the BUI method showed saturation with a Km value of 5.8 mM, and is specific for the reduced form of glutathione, GSH.[81,82] Significant transport of GSH in intact form was also demonstrated by the brain perfusion method.[83] When mRNA obtained from brain capillaries was injected into oocytes, GSH transport activity, via both sodium-dependent and -independent mechanisms, was observed with a Km for sodium-dependent transport of 0.4 mM, demonstrating that the transporter protein is present at the BBB.[84] Furthermore, cultured MBEC4, a model cell line for mouse brain capillary endothelial cells, exhibited sodium-dependent transport activity for GSH in a fraction rich in apical membrane.[85] These observations suggest that GSH is transported in the blood-to-brain direction by a specific sodium-dependent transporter, as well as a sodium-independent one.

Toyobuku *et al.*[86] examined the feasibility of heterologous transduction of a transporter gene to the BBB. As peptides have multiple biological actions in the brain, they are potentially valuable as neuropharmaceuticals in the treatment of various disorders, such as Alzheimer's disease and depression. Delivery of peptide drugs to the brain, however, is a major challenge, because distribution of peptides to the brain is generally very low owing to the presence of the BBB. Toyobuku *et al.*[86] constructed a recombinant adenovirus vector encoding PEPT1 and transduced the transporter into the BBB of rats by carotid artery injection of the vector. Heterologous expression of PEPT1 at the BBB successfully increased the brain distribution of a model substrate, cefadroxil, of PEPT1.[86] Malignant gliomas are the most common primary neoplasms of the CNS. The prognosis for high-grade malignant gliomas remains bleak; survival is generally less than 1 year. Because bestatin, the substrate for PEPT1, was reported to induce apoptosis in glioma cells,[87] this system may be applicable to the treatment of malignant glioma.

CONCLUSIONS

Clarification of the transport mechanisms of drugs across the BBB is important to improve the efficacy of CNS-active drugs or to reduce the CNS toxicity of drugs that are active in peripheral tissues. Recent molecular identification and functional analyses of carrier-mediated transport systems for drugs, as well as endogenous compounds, at the BBB show that brain capillary endothelial cells are equipped with various membrane transport mechanisms in both blood-to-brain (influx) and brain-to-blood (efflux) directions to maintain homeostasis. In addition to the transport mechanisms covered in the present review, there must be other influx transport processes. Accordingly, utilization of influx transporters is expected to be useful in delivering many kinds of hydrophilic drugs into the brain. Furthermore, like eliminating organs such as liver and kidney, the BBB contains multiple efflux transporters, including P-gp and MRP or related transporters. Because various drugs are excluded from the brain by such transporters, prevention of efflux transport by the use of specific inhibitors is also an attractive approach to improve the brain distribution of drugs. It is essential to identify all the transporters and to characterize them functionally to provide a basis for strategies to regulate drug disposition in the brain.

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