DRUG DISPOSITION AND TARGETING:

TRANSPORT ACROSS THE BLOOD BRAIN BARRIER

Bertrand Rochat and Kenneth L. Audus

Department of Pharmaceutical Chemistry
School of Pharmacy
The University of Kansas
Lawrence, Kansas 66047

Address correspondence to:
Dr. Kenneth L. Audus
Department of Pharmaceutical Chemistry
The University of Kansas
2095 Constant Ave., 236B Simons
Lawrence, KS 66047
Phone: (785)864-3609
Fax: (785)864-5736
e-mail: audus@smissman.hbc.ukans.edu

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TABLE OF CONTENTS

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1. INTRODUCTION

The concept of the blood-brain barrier (BBB) was developed late in the last century on the basis that certain dyes did not accumulate in the brain, in contrast to other organs. Just after the middle of this century, research demonstrated that the BBB resides in the endothelium of the brain microvessels, represented a significant surface area of exchange between blood and brain (around 12 m^2), and was, however, the primary barrier to drug and peptide entry into the brain (Pardridge, 1994). The modern view of the BBB may be regarded as a dynamic barrier where many of its characteristics potentially may be up or down regulated in normal as well as in disease states.

Several specific characteristics of the brain endothelium participate in formation of this barrier, including anatomical or morphological features illustrated in Figure 1. In contrast to peripheral endothelial microvessels, the endothelium comprising the BBB has no fenestrations and a reduced number of pinocytic vesicles suggesting that bulk transfer and transcytotic mechanisms for moving macromolecules are not well elaborated at the BBB. However, as reviewed below, specific, unidirectional movement of a limited number of macromolecules does occur at the BBB (Broadwell and Banks, 1993). The cells also exhibit the presence of unusually tight intercellular junctions (Reese and Karnovsky, 1967; Brightman and Reese, 1969) which effectively "seal" the apposing intercellular membranes of the endothelial cells together and confer a high membrane resistance (Crone and Olesen, 1982). Thus, the passive movement of macromolecules and hydrophilic compounds paracellularly is effectively blocked and forces substances to undergo transcytotic transport. The brain endothelial cells also contain many mitochondria, indicating that the barrier between blood and brain can contribute to biotransformation of xenobiotics (Pardridge, 1983, Ghersi-Egea et al., 1995; Minn et al.,

1991). Cells surrounding the brain endothelium (i.e., neurons, astrocytes and pericytes), as well as the basal lamina, seem to contribute to the stability and the barrier characteristics of the brain endothelial cells. These cell-cell interactions appear to be via the secretion of trophic factors rather than direct physical contact (Joo, 1996; Graguera et al., 1993; Claudio et al, 1995; Ment at al. 1995).

In order to understand the transport mechanisms of the BBB, *in vivo*, *in situ*, and *in vitro* systems have been developed and are now in use to explore the biochemical and molecular basis of the BBB. While *in vivo* or *in situ* studies can provide an indication of the delivery potential and pharmacological consequences in animal models, the *in vitro* systems (i.e., isolated brain capillaries, cell cultures, etc.) offer promising tools in order to characterize properties specific to brain endothelial cell transport and metabolism at the cellular, biochemical, and molecular levels (Audus et al., 1996). Addressing the application, advantages, and disadvantages of different experimental systems is beyond the scope of this chapter and interested readers are directed to recent reviews (Audus et al., 1996; Smith, 1996).

Further discussions in this chapter will survey the membrane transport processes that currently allow drugs and macromolecules to permeate the BBB. A number of transcellular transport systems in brain endothelia exist for the transport of vital nutrients and waste products between the blood and the central nervous. The presence of an efflux pump, the multidrug resistant gene product 1 (MDR1), an overexpressed P-glycoprotein, compounds the picture of the BBB and is included here as a significant problem that must be overcome in many drug delivery strategies. We also include mention of several less well-characterized systems which might contribute to transporter-targeted delivery strategies in the future. We hope this discussion might

stimulate researchers to develop a better knowledge of natural transport systems at the BBB and underscore more strongly their possible primordial role in drug delivery and therapeutic efficacy.

2. BLOOD BRAIN BARRIER TRANSPORT

2.1. Carrier-Mediated Transport

Carrier-mediated transport may be described as either facilitative which is saturable, stereoselective, and energy-independent, or active which is saturable, stereoselective and directly dependent on energy expenditure by the cell. Several carrier mechanisms have been described at the BBB and now include both active and facilitative types as summarized in Table 1. Only a few of these carriers have been extensively characterized and cloned. For some, such as the neutral amino-acid carrier, their utility in drug delivery to the brain is well known.

2.1.1. Carriers and efflux systems for low molecular weight molecules

At least five D-glucose transporters have been described (Thorens, 1996). From the earliest studies, it appeared that D-glucose crossed the BBB easily and stereospecifically, thanks to a high density of facilitative type transporters. Discrepant results have been published concerning the reported affinity constants of glucose for "its" transporter, GLUT-1 (Audus et al., 1992), and could arise from the overlapping specificities of the various transporters. A recent study has described a sodium-dependent, active transport of glucose at the BBB that apparently functions in parallel to the facilitative GLUT-1 transporter (Nishizaki et al. 1995).

The GLUT-1 transporter is inhibited by barbiturates (Honkanen et al., 1995) and its gene expression can be controlled by cerebrolysin (Boado, 1995). Although there is a relatively low affinity of the substrate D-glucose for the GLUT-1 carrier (K_m in the mM range), no drugs have been shown to be competing substrates. There are a few indications that GLUT-1 can transport substrates other than D-glucose. As examples, glucosylation of few peptides has been shown to increase their transport across the BBB and the evidence seemed to indicate that GLUT-1 was responsible for this transport (Polt et al., 1994). Additionally, the oxidized form of ascorbic acid has been shown to be transported by the GLUT-1 carrier (Agus et al., 1997). Attempts have also been made to couple chemotherapeutics with glucose (e.g., glucose-chlorambucil derivatives) only to find that, while the agents inhibit the GLUT-1 transporter, the agents are not transported (Halmos et al., 1996). Despite some positive observations, GLUT-1 does not appear to be an obvious carrier of choice for the drug delivery into the brain.

Saturable and bidirectional carriers for monocarboxilic acids have been decribed at the BBB and are responsible of the transport of L-lactate, L-acetate and L-pyruvate, valproic acid, salicylic acid and saturated fatty acids (Conn et al., 1983; Adkison and Shen, 1996; Gerhart et al., 1997). In general, these carriers have been more studied in intestinal cells like Caco-2 cells and are better known in that cell type (Tamai et al., 1995; Takanaga et al.,1994). Drugs such as valproic acid inhibit the uptake of short-chain monocarboxylic acids such as lactate, acetate and pyruvate at the BBB. However, the uptake of valproic acid is not inhibited by short-chain monocarboxylic acids. Rather, the passage of valproic acid across the BBB appears to occur through a medium-chain fatty acid transporter (Adkinson and Shen, 1996). Therefore, it appears that multiple carriers for monocarboxylic acid compound are probably expressed at the BBB and

show overlaps in substrate selectivity. Specific antibodies as well as molecular probes may allow a better characterization of the transporters of monocarboxylic acid compounds and eventually demonstrate their importance in the delivery of drug across the BBB.

Neutral amino-acid carriers have proven to be capable of transporting many amino acids, drugs and endogenous compounds with similar stuctures. Leucine, cysteine, serine, alanine, phenylalanine, L-dopa, L-tryptophan, the alkylating agent melphalan, the antiepileptic drug gabapentin, the muscle relaxant baclofen, the neurotoxin beta-N-methylamino-L-alanine are all examples of agents shown to be substrates of neutral amino-acid carriers (Begley, 1996, De Boer and Breimer, 1994; Smith et al. 1992; Oldendorf and Szabo, 1976). Various forms of neutral amino-acid carriers have been described (Sanchez-del-Pino et al., 1995; Zerangue et al., 1996). For instance, the carrier for large neutral amino-acids (LNAA) mainly transports phenylalanine with a high affinity (K_m about 10 μM) and is sodium-independent. Since LNAA carrier is saturated under normal conditions by neutral amino acids present in blood, substrate competition does occur *in vivo* and does impair delivery of lower affinity substrates (Shulkin et al., 1995). The large neutral amino acid carriers, located on the luminal and abluminal membranes of the brain endothelial cells, appear to be subject to regulation by endogenous compounds such as oxoproline (Lee et al., 1996) and vasopressin (Reichel et al., 1996).

In contrast to dopamine, L-dopa is transported across the BBB by the LNAA carrier and is readily biotransformed into the brain to dopamine. At present, the pro-drug L-dopa represents the best example of drug delivery mediated by a facilitated amino acid carrier at the BBB. Alkylating agents, mephalan and the nitrogen mustard, DL-NAM,

have also shown to be substrates of LNAA carrier at the BBB (Begley, 1996; and Takada et al., 1992). The affinity of DL-NAM for the LNAA carrier is 100 times higher than mephalan because the addition of a naphtoic side chain. LNAA carriers show stereospecific activity for the S-isoforms of phenylalanine derivatives prepared as NMDA receptor antagonists. As an example, L-4-chlorokynurenine might be a useful prodrug for brain delivery of Glycine-NMDA receptor antagonist since it is rapidly transported across the BBB by the LNAA carrier and enzymatically converted in brain parenchyma to the therapeutic form, 7-chlorokynurenic acid (Hokari et al., 1996). The cytotoxic agent acivicin causes neurotoxicity due to its broad penetration into the brain, largely mediated by LNAA carriers. Thus, acivicin derivatives showing a lower affinity for LNAA carriers could exhibit a lower CNS toxicity (Chikhale et al., 1995a).

Other neutral amino-acid carriers (i.e, types A, ASC and N) showing more selectivity for small neutral amino-acid and a sodium dependence, have been described at the BBB (Zerangue et al. 1996; Sanchez-del-Pino et al., 1995). These amino acid carriers do have overlaps in amino acid recognition. For instance, the BBB transport of L-glutamine, mainly found in the cerebrospinal fluid, has been investigated and there has been an indication that two neutral amino-acid carriers were involved in the L-glutamine transport: a Na-independent system L and a Na-dependent system N (Keep and Xiang, 1995). Taking into consideration all of these examples, the neutral amino acid carriers continue to have the potential for brain delivery of therapeutic agents. Nevertheless, progress has yet to be made regarding the definition of the broad functions, affinities, and the specificities of all of BBB neutral amino-acid carriers.

Basic amino-acid and acidic amino-acid transporters have been shown to transport asparagine, lysine and aspartate and glutamate across the BBB (Oldendorf, 1971; Benrabh

and Lefauconnier, 1996). Amine transporters are able to transport choline across the BBB (Galea and Estrada, 1992). The existence of nucleoside transporters has also been demonstrated (Joo, 1996). These latter carriers are able to transport the purines adenosine, guanosine, inosine and the pyrimidine uridine across the BBB (Cornford and Oldendorf, 1975; Thomas and Segal, 1997). Thiamine and thyroid transporters are able to transport thiamine, vitamins such as folates and ascorbic acid, and T3 across the BBB, respectively (Greewood et al., 1982; Joo, 1996). Once more, molecular and biochemical characterizations of these transporters will allow a better understanding of their potential for exploitation in drug delivery.

Many drugs have been shown to cross the brain endothelial cells poorly in spite of their favorable coefficient partitions (i.e., relatively lipophilic compounds). In fact, many drugs and peptides are pumped outside the cells by active transporters which are present in many cell types (Lum and Gosland, 1995). These transmembrane pumps contribute to the resistance to anticancer agents and probably psychotropic drugs in both normal and tumor tissues. P-glycoprotein (P-gp) is actually the most documented efflux pump in resistant cell lines as well as in normal tissues (Stein, 1997; Lum and Gosland, 1995) and is responsible for the multdrug resistance type 1 (MDR1) phenotype *in vitro* as well as *in vivo* (Lum and Gosland 1995; Huang et al., 1997). Carcinogens and various xenobiotics are capable of co-inducing the both expression of P-gp, as well as some cytochrome P-450 isozymes, in normal tissues as well as in cancer resistant cell lines (Burt and Thorgeirsson, 1988; Thorgeirsson et al., 1991). Inhibition of the efflux mechanism by the so-called reversing agents allows an increased accumulation of P-gp substrates inside the cells (Wigler, 1996). The P-gp of the BBB has been shown to play a sometimes dominant role in the efficacy of many drugs in the central nervous system

(Schinkel et al., 1994; 1996). In humans, the activity of the brain P-gp is about one-fourth that expressed in the gut (Silverman and Schrenk, 1997).

The number and diversity of drugs shown to be substrates of the P-gp suggests that, like cytochrome P-450, the specificity of P-gp is very broad (Gottesman and Pastan, 1993). In the near future, other drug efflux systems (MDR-like pumps) are likely to be discovered and characterized at the BBB. Clearly, not all drugs have the same affinity for MDR1 present at the BBB, MDR1 systems do vary somewhat among species (Schinkel et al., 1994; 1996), and there are some indications that MDR1 may vary in localization at the blood-brain barrier, particularly in the human, where there is conflicting evidence of MDR1 localization from the typical luminal membrane (Stewart et al., 1996) to an abluminal and astrocytic foot location (Pardridge et al., 1997). The multidrug resistance protein, MRP, which has been described in cancer-resistant cell lines as well as normal tissues (Kavallaris, 1997; Berger et al., 1997; Barrand et al., 1997), is expressed and functional at the BBB (Miller et al., 1997). In cancer cell lines, other MDR transporters, different from the MDR1 and MRP, have already been identified (Izquerdo et al., 1996; Huang et al., 1997; Lee et al., 1997).

It appears that, in most instances, lipophilic drugs are substrates of an ATP-dependent efflux pump, MDR1, an overexpressed P-glycoprotein (P-gp) localized at luminal membranes of the brain endothelial cells. Structurally diverse compounds are able to inhibit, sometimes in a competitive manner, the P-gp activity. Known substrates include vinblastine, reserpine, verapamil, trifluoperazine, amiodarone, daunomycin, progesterone, propafenone, and quinidine (Drion et al., 1996; Wigler, 1996; Kavallaris, 1997; Stein, 1997).

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As transporters, drug efflux systems remain important BBB targets that must be neutralized to realize delivery of a wide variety of therapeutic agents. Cyclosporine A is able to inhibit completely the P-gp activity (Wigler, 1996) and the cyclosporine D derivative, SDZ PSC 833, has also been used *in vivo* and *in vitro* and show a potent P-gp inhibition. The pipecolinate derivative of cyclosporine D, VX-710, which is able to inhibit P-gp, showed a stronger inhibition of the MRP activity effect than either verapamil or cyclosporin A (Germann et al., 1997). The ATPase portion of the P-gP can be inhibited by bafilomycin A1. P-gp ATPase activity can be stimulated by colchicine, verapamil and trifluoperazine (Sharom, 1995). The phospholipids surrounding the P-gp ATPases have been identified and could play an important role in its activity. It has been suggested that the inhibition of the P-gp activity by so-called fluidizers could occur by perturbing the lipid intregrity around the P-gp and its ATPase (Drori et al, 1995).

2.1.3. Peptide carriers

A limited but growing body of research has been focused on saturable carriers for selected peptides at the BBB. These carriers are both unidirectional, either transporting peptides out of the brain or into the brain, and bidirectional (Banks and Kastin, 1996). Carrier systems for small peptides at the BBB are summarized in the Table 2. At present, there appear to be no carriers of peptides larger than 10 amino-acids at the BBB and those larger peptides would be expected to undergo transcytosis (Broadwell and Banks, 1993). At present, many of these carrier mechanisms lack sufficient characterization to propose precise roles for the mechanisms in drug delivery schemes.

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Several small peptides have been shown to be apparent substrates of P-gp efflux at the BBB (Sharma et al., 1992; Sarkadi et al., 1994; Chikhale et al., 1995b). However, independent of P-gp, the unidirectional transport of peptides such as corticotropin-releasing hormone (CRH) have been shown to be mediated by an active transporter and dependent on calcium channels (Martins et al., 1996 and 1997). Similary, a number of other small peptides also have distinct, independent transport systems to move the peptides from the brain into the systemic circulation. Among these are small tyrosinated peptides, Met-enkephalin and Tyr-MIF-1 (Banks and Kastin, 1997; Banks et al., 1994). The transporter for these latter peptides is notable for being sensitive to ethanol which can enhance the brain Met-enkephalin concentration (Plotkin et al., 1997). A separate efflux system has also been described for RC-160, a somatostatin analog (Banks et al., 1994) and arginine vasopressin (Begley, 1996).

In *in vivo* experiments, D-[Ala^1]-peptide T amide is transported from blood to brain by a saturable system (Barrera et al., 1987). Using intraventricular injections of peptide T, the transport out of the brain was not saturable indicating a specific localization of the carrier at the membrane of the endothelial cells. Studies employing intravenous injection of radiolabelled leptin revealed the presence of a saturable blood-to brain transporter (Banks et al., 1996). Similar to peptide T, no saturable transport of radiolabelled leptin out of the brain was observed. Neurotensin is another representative small peptide that also has shown only unidirectional transport into the brain (Banks et al., 1995).

Some of the opioid peptides, including deltorphins, cross the BBB rather effectively and experiments performed with isolated bovine brain microvessels indicate presence of a naloxone-sensitive carrier (Fiori et al., 1997). Preloading the microvessel

preparations with L-glutamine can also transiently stimulate of deltorphin uptake suggesting some interrelationships with amino acid transport at the BBB. The delta opioid receptor-selective [D-Penicillamine-2,5] enkephalin (DPDPE) has been shown to penetrate the brain by a saturable system (Thomas et al., 1997). The uptake system for DPDPE, which does not involve the large amino acid transporter, has yet to be fully characterized. Transport of leucine-enkephalin across the BBB has also been demonstrated *in vivo* and exhibited a saturable mechanism (Zlokovic et al., 1987). The potential relationships among the carriers for opioids at the BBB have not been resolved. The possibility obviously exists, however, for the design and development of opioid peptide therapeutics that target BBB carriers and achieve improve brain delivery (Thomas et al., 1997).

The last group of peptide carriers to be mentioned facilitate peptide distribution across the BBB in both directions. Bidirectional and saturable transporters exist for peptides such as luteinizing hormone-releasing hormone (LHRH; Barrera et al., 1991) and Interleukin-1 alpha (Banks et al., 1989). Different transporters, sodium dependent or independent, have also been identified for glutathione (Kannan et al., 1996).

2.2 Transcytosis

Macromolecules may cross the brain microvessel endothelial cells by transcytosic mechanims mediated by luminal surface receptors (Audus et al., 1992; Broadwell and Banks, 1993). While the role of plasmalemmal vesicles as transcytotic carriers through the endothelium was postulated in the 1960's but the use of a combination of a non-transportable moiety with a natural macromolecule, a chimeric drug delivery system, recognized by membrane receptors mediating transcytosis at the

BBB was not proposed until the late 1980's (Pardrige et al., 1987). Subsequently, several receptor systems mediating transcytotic processing through the BBB have been described and are summarized in the Table 3. In several instances, these transcytotic systems have been explored as mechanisms to target and facilitate the delivery of therapeutic entities to brain capillary endothelial cells and eventually the brain.

A high rate of transcytosis of insulin in brain capillary endothelial has been reported *in vivo* and suggests that the insulin receptor could be an effective target for drug delivery into the brain (Duffy and Pardridge, 1987; Podulso et al., 1994). Related peptides, insulin-like growth factor I and II, also show significant brain levels following injection into the carotid artery of rats (Rheinhardt and Bondy, 1994). Native insulin has been used as a carrier of horseradish peroxidase, a protein which crosses the BBB very slowly (Fukuta et al., 1994). The difficulty in using native insulin as carrier is the associated hypoglycemia. For this reason, insulin fragments have also been examined as carriers and have shown some promise (Fukuta et al., 1994).

Transferrin is another natural polypeptide that like, insulin, crosses the BBB by a receptor-mediated system (Broadwell et al., 1996). While native transferrin has also been considered as a potential chimeric carrier, most current efforts focus on the use of monoclonal antibodies directed at specific receptor systems. Monoclonal antibodies generated against transferrin or insulin receptors have been shown to cross the BBB (Friden et al., 1996; Pardridge et al., 1995b). Due to its rapid transcytosis the insulin receptor monoclonal antibody has been proposed as an effective drug delivery vector and potential diagnostic tool and has been demonstrated in a primate model (Wu et al., 1997). The murine OX26 monoclonal antibody to rat transferrin receptor was also successfully used as vector in order to increase the delivery of brain-derived

neurotrophic factor and polyamide nucleic acids across the BBB (Pardridge et al., 1994; Pardridge et al., 1995a). On comparison, horseradish peroxidase and native transferrin complexes do cross the BBB more efficiently than horseradish peroxidase and OX26 antibody complexes. After i.v. injection in rat, some differences in intracellular and extracellular distributions in brain were observed indicating that fates in the central nervous system of delivery systems employing native transferrin and monoclonal antibodies to the transferrin receptor will differ (Broadwell et al., 1996). Indications are that both insulin and transferrin receptor targeting monoclonal antibodies could be useful for non-invasive delivery of therapeutic entities to the brain.

Other endogenous substances appear to undergo receptor-mediated transcytosis at the BBB and their receptors may be considered potential delivery targets. Low density lipoprotein (LDL) is specifically transcytosed across brain capillary endothelial cells (Dehouck et al., 1997). LDL is transcytosed by a receptor mediated system which can be upregulated by the depletion of cholesterol in the astrocyte:endothelial cell co-cultures. A receptor-mediated transport process has also been reported for the amyloid beta-protein suggesting that sources outside the nervous system could contribute, at least partially, to the cerebral A beta-amyloid deposits seen in Alzheimer's patients (Podulso et al., 1997). Finally, leptin, a protein synthesized by adipose tissue as signal of satiety, penetrates the BBB via trancytosis mediated by specific receptors (Golden et al., 1997).

Several proteins have been shown to undergo adsorptive transcytosis at the BBB, however, the precise specificity and therefore targeting potential is not well developed for this group. A recent report indicates that the C-terminal structure and basicity of peptides seems to be important for the saturable uptake by an adsorptive-mediated endocytosis system at the BBB (Tamai et al., 1997). The endogenous cationic protein,

histone, is also capable of crossing the BBB via an adsorptive transcytotic system (Pardridge et al., 1989). Natural proteins such as immunoglobulin G and albumin have been shown to cross the BBB with low permeability surface area products, approximately about 25 and 330 times less than the BBB permeability of transferrin and insulin, respectively (Podulso et al., 1994). Although albumin transcytosis apparently does not involve a receptor-mediated system (Vorbrodt and Trowbridge, 1991), the more rapid rate of immunoglobulin G transfer suggests the potential of a transport mechanism (Podulso et al., 1994). Cationionized albumin, with a greater pI than the native albumin, enters more readily into the brain and seems to be a tool to study the adsorptive-mediated endocytosis at the brain endothelial cells (Kumagai et al., 1987). Selected lectins undergo transcytosis at the BBB. Complexes of horseradish peroxidase and wheat germ agglutinin, for example, penetrate the brain 10 times higher than horseradish peroxidase alone via a probable non-specific adsorptive (Villegas and Broadwell, 1993; Banks and Broadwell, 1994).

It appears that the complexing of a therapeutic agent with a carrier recognized by brain capillary specific endothelial cell receptors can provide a choice for some drug delivery strategies since the complex may, in some instances such as transferrin, selectively target the BBB (Friden, 1994). Due to the probable differing fates, structures, and capacities for complexation of the carrier molecules, a single carrier system will not be practical. Thus, the precise determination of chemical structures recognized by BBB receptors mediating trancytosis should continue to be a fruitful area of research in the future.

3. SUMMARY AND FUTURE PERSPECTIVES

The restrictive permeability properties of the BBB remain a challenge to providing sufficient drug delivery into the central nervous system. Basic research in the past few decades surveyed here has revealed, however, that the BBB exhibits an array of specific transport systems for both conventional low molecular weight nutrients and hormones, as well as limited selection of systems for natural peptides and proteins. Future success in exploiting these transport processes is now dependent, to some degree, on incorporating the advances in cellular, biochemical and molecular biology into thinking about targeting natural transporters to facilitate drug delivery into the central nervous system.

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Figure Captions

Figure 1. Anatomical and morphological characteristics of endothelium of the blood-brain barrier and the peripheral vasculature. (Reprinted from Audus and Borchardt, 1991 with permission from Springer-Verlag GmbH & Co. KG, Heidelberg.)

Rochat, B. and Audus, K.L. (1999) Drug disposition and targeting: Transport across the blood-brain barrier. Pharm. Biotechnol. 12, 181-200. PMID: 10742975. Publisher's official version: <http://dx.doi.org/10.1007/0-306-46812-3_6>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Table 1. Blood-brain barrier transport systems for nutrients and low molecular weight organic molecules.

Carrier	*Type*	*Substrate*	*Direction*	*Reference*
A amino acid	active	alanine	brain-to-blood	Komura et al., 1996
Acidic amino acid	facilitated	glutamate	blood-to-brain	Benrabh and Lefauconnier, 1996
Amine	active	choline		Galea and Estrada, 1992
Basic amino acid	facilitated	arginine		Oldendorf, 1971
Basic organic molecules		mepyramine		Yamazaki et al., 1994
Biotin	facilitated	biotin	bidirectional	Shi et al., 1993
Carnitine	facilitated	carnitine	brain-to-blood	Mroczkowska et al., 1997
Medium-chain fatty acid		valproic acid		Adkison and Shen, 1996
Hexose	facilitated	glucose	bidirectional	Pardridge et al., 1990
Monocarboxylic acid	active	lactate	bidirectional	Conn et al., 1983
Large neutral amino acid (LNAA)	facilitated	phenylalanine	bidirectional	Oldendorf and Szabo, 1976
Pantothenic acid	facilitated	pantothenic acid	blood-to-brain	Spector et al., 1986

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Purine	facilitated	adenine		Cornford and Oldendorf, 1975
Pyrimidines	facilitated	thymidine		Thomas and Segal, 1997
Taurine	active	taurine	bidirectional	Tamai et al., 1995
Thiamine	facilitated	thiamine		Greenwood et al., 1982
Thyroid hormone	facilitated	thyroid hormone		Pardridge, 1979

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Table 2. Blood-brain barrier peptide transport systems.

Carrier	*Type*	*Substrate*	*Direction*	*Reference*
Corticotropin-releasing hormone (CRH)	active	CRH	brain-to-blood	Martins et al., 1996
D-[Ala^1]-peptide T-amide	facilitated	D-[Ala^1]-peptide T-amide	blood-to-brain	Barrera et al., 1987
Deltorphin	facilitated	deltorphin	blood-to-brain	Fiori et al., 1997
[D-Penicillamine^{2,5}]-enkephalin (DPDPE)		DPDPE	blood-to-brain	Thomas et al., 1997
Glutathione	active	glutathione	bidirectional	Kannan et al., 1996
Cytokines		interleukin-1	bidirectional	Banks et al., 1989
Leucine enkephalin		leucine enkephalin		Zlokovic et al., 1987
Luteinizing hormone-releasing hormone (LHRH)	facilitated	LHRH	bidirectional	Barrera et al., 1991
Neurotensin	facilitated	neurotensin		Banks et al., 1995
Somatostatin		somatostatin	brain-to-blood	Banks et al., 1994

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Tyrosinated peptides	active	methionine enkephalin	brain-to-blood	Banks and Kastin, 1997
Vasopressin	facilitated	vasopressin	brain-to-blood	

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Table 3. Blood-brain barrier macromolecule endocytic and transcytotic systems.

Carrier	*Type*	*Reference*
Amyloid ß-protein	receptor	Poduslo et al., 1997
Basic peptides	adsorptive	Tamai et al., 1997
Histone	adsorptive	Pardridge et al., 1989
Immunoglobulin G		
Albumin	adsorptive (?)	Poduslo et al., 1994
Insulin	receptor	Duffy and Pardridge, 1987
Insulin-like growth factor	receptor	Reinhardt and Bondy, 1994
Low density lipoprotein	receptor	Dehouck et al., 1997
Lectins	adsorptive	Villegas and Broadwell, 1993
Leptin	receptors	Banks et al., 1996
Modified albumins	adsorptive	Kumagai et al., 1987
Transferrin	receptor	Broadwell et al., 1996; Bradbury, 1997