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RECENT ADVANCES IN BLOOD-BRAIN BARRIER TRANSPORT

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INTRODUCTION

The concept of the blood-brain barrier (BBB) is gradually changing from one of a passive, relatively immutable structure to that of a dynamic membrane interface between blood and brain that is regulated by the brain itself and is vital to brain function (1). The BBB allows the brain to communicate with the internal environment in blood, just as the five senses allow for brain's communication with the external environment.

The blood-brain barrier is found in all vertebrates (2), and it is found in the first trimester of human fetal life (3). The BBB arises from epithelial-like tight junctions that virtually cement adjoining capillary endothelium together in the brain microvasculature (4). Thus, there are no pores in brain capillaries. Circulating small molecules, peptides, or drugs, which normally freely gain access to the interstitial space in nonbrain organs, are barred from brain interstitial space—unless these molecules have an affinity for one of the numerous specialized enzymelike transport systems localized within the BBB (5). These transport systems are localized on the lumenal, or blood side, of the BBB. This location allows for movement from blood to the capillary endothelial cytoplasm. They are also present on the antilumenal border, or brain side, of the brain capillary endothelium, which allows for movement from the endothelial cytoplasm to brain interstitial space (6–8).

The ultimate aim of blood-brain barrier research is to describe the capillary endothelial cell transport processes within the context of molecular cell physiology. As I discuss in the last section of this review, an understanding of

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the cellular physiology of BBB transport processes invariably leads to the design of new strategies for drug delivery through the BBB (9). Prior to the discussion of drug delivery systems, I discuss newer aspects of our understanding of the cell biology of the BBB, as well as the diversity of BBB transport processes. These transport mechanisms fall into at least three major categories: (*a*) carrier-mediated transport of nutrients, thyroid hormones, or drugs; (*b*) receptor-mediated transport of peptides, plasma proteins, or viruses; and (*c*) plasma protein–mediated transport of plasma protein-bound substances, such as steroid hormones, free fatty acids, or drugs.

CELL BIOLOGY OF THE BLOOD-BRAIN BARRIER

Recent studies with transplant paradigms have provided support for the model that the genes encoding the unique biochemical characteristics of the brain capillary endothelium are activated by trophic factors. These factors are secreted by the brain itself and, most likely, by astrocytes. Using a quail-chick transplant paradigm, Stewart & Wiley (10) transplanted embryonic gut to embryonic brain prior to organ vascularization. The transplanted gut was perfused by capillaries of brain origin. However, those capillaries within the gut-transplanted tissue did not have the characteristics of brain capillaries (e.g. exclusion of vital dyes such as trypan blue following systemic administration). Conversely, when embryonic brain was transplanted to embryonic gut prior to organ vascularization, the transplanted brain was perfused by capillaries of gut origin. These capillaries had the usual properties of the BBB. This suggests that the unique morphological characteristics of the brain capillary endothelium are induced by factors secreted by the brain itself. Subsequent studies show that the astrocyte is the most likely cellular origin of the putative BBB trophic factors (11). A major challenge to future BBB research is the isolation and characterization of these putative trophic factors, which are presumably peptides. Methods for culturing brain capillary endothelium, either in primary cultures or as cell lines, provide an experimental paradigm that may lead to the identification of these factors (12, 13).

The glial induction model regarding the maintenance of the BBB has a clinical analogue in the area of brain tumors. Well-differentiated primary glial tumors of brain have an intact BBB and do not show contrast enhancement on CT scans (14). The BBB is intact, presumably because of the continued secretion of the glial factors. Conversely, poorly differentiated brain tumors or tumors of nonbrain origin, e.g. meningioma or metastatic tumors, have a porous BBB, as revealed by contrast enhancement on CT scans. These tumors lack the BBB due to the presumed absence of glial cells and the continuous secretion of glial trophic factors.

CARRIER-MEDIATED TRANSPORT

Specific carrier-mediated transport systems have been described for numerous classes of nutrients, as well as for the thyroid hormones (Table 1). The most abundant transport system in the BBB is the glucose carrier (6, 15, 16). The BBB glucose carrier has recently been photoaffinity-labeled using ^{3}H-cytochalasin B. The molecular mass of this transport system is 53,000 daltons (17, 18). The human and rat sodium-independent glucose transporters have been cloned, and the DNA sequences of these genes are 98% homologous (19, 20). Using a cDNA to the human erythrocyte glucose tranporter, Flier et al (21) demonstrated that the 2.8-kilobase (kb) transcript that encodes for the glucose transporter is most abundant in the brain capillary, as compared to any of the tissues analyzed thus far. Morevover, Dick & Harik (22) showed that there are tenfold more glucose transporter cytochalasin B binding sites in the brain capillary than on brain synaptosomes. The expression of the BBB glucose transporter gene appears to be regulatd by the ambient concentration of glucose. For example, chronic hyperglycemia leads to a down-regulation of the BBB glucose transporter (23, 24), and chronic hypoglycemia leads to up-regulation of the activity of the BBB glucose transporter (25).

The kinetic constants shown in Table 1 for the nutrients or thyroid hormones were determined with the carotid artery single-injection technique (26). However, recent studies show that K_m estimates for the neutral or basic amino acid transport systems determined with this technique are approximately two- to threefold higher than the actual values (27). This overestimation is caused by the efflux of unlabeled amino acid from brain into the injection bolus as it traverses the cerebral microcirculation. More accurate estimates of amino acid transport at the BBB can be obtained with the carotid artery infusion technique (28), which employs a rate of cerebral blood flow that is

Table 1 Blood-brain barrier nutrient and thyroid hormone carriers[a]

Carrier	Representative substrate	K_m (μM)	V_{max} (nmol min^{-1}g^{-1})
Hexose	glucose	11,000 ± 1,400	1,420 ± 140
Monocarboxylic acid	lactic acid	1,800 ± 600	91 ± 35
Neutral amino acid	phenylalanine	26 ± 6	22 ± 4
Amine	choline	340 ± 70	11 ± 1
Basic amino acid	arginine	40 ± 24	5 ± 3
Nucleoside	adenosine	25 ± 3	0.75 ± 0.08
Purine base	adenine	11 ± 3	0.50 ± 0.09
Thyroid hormone	T_3[b]	1.7 ± 0.7	0.19 ± 0.08

[a] From (81, 82).
[b] T_3 = triiodothyronine.

approximately tenfold the normal value. Owing to the extremely short transit time with this procedure, the efflux of unlabeled amino acid from brain into the infusate is minimal. Therefore, the affinity of the BBB neutral amino acid transport system for circulating neutral amino acids is even higher than previously thought (27). This increased affinity is the basis for the unique vulnerability of the central nervous system (CNS) to competition at BBB transport sites caused by selective hyperaminoacidemia such as hyperphenylalaninemia (29). The inhibition of brain protein synthesis caused by hyperphenylalaninemia is reversed by the administration of other large neutral amino acids that compete with phenylalanine for transport on the BBB neutral amino acid carrier (30). Mild hyperphenylalaninemia is now possible on a large scale, owing to the widespread use of the new nonnutritive dipeptide sweetener, aspartame (aspartylphenylalanine methyl ester) (31). The ingestion of 5 servings of aspartame per 50 pound body weight per day results in a doubling of the plasma phenylalanine in normal individuals, and a tripling of the plasma phenylalanine in phenylketonuric heterozygotes, e.g. from 50 μM to 150 μM (32). This increased concentration may cause a selective saturation of the BBB neutral amino acid carrier in humans, since the K_m of phenylalanine transport at the human BBB is low, similar to that of the rat. The K_m of phenylalanine transport into isolated human brain capillaries, 22 $\pm$ 7 μM, is nearly identical to that of phenylalanine transport into isolated rat brain capillaries, 11 $\pm$ 2 μM (29). Saturation of the carrier by selective increases in the plasma phenylalanine concentration to 150 μM may lead to a depression in the brain uptake of other neutral amino acids such as tryptophan or tyrosine, which are precursors to the monoamines, serotonin and the catecholamines, respectively (33). Whether a threefold elevation in brain phenylalaine, e.g. from 50–150 μM, is deleterious to the human brain remains to be seen, but it is now clear that there is a need for clinical studies in this area, given the widespread use of aspartame as a sweetener.

The basis for the stereospecific differences in the biologic potency between the D- vs L-isomers of triiodothyronine (T_3) has been an enigma in thyroid hormone physiology. The L-isomer is three- to tenfold more active than the D-isomer (34), yet the nuclear T_3 receptor, which mediates much of thyroid hormone action, is not stereospecific (35). Recent studies of BBB transport of T_3 may shed light on this area. The BBB T_3 carrier is sharply stereospecific, with the L-T_3-isomer having a ninefold greater affinity for the transport system than the D-isomer (36). Moreover, a recent study shows that the intracerebral administration of T_3 to hypothyroid rats increases heart rate more than the same dose of T_3 administered intravenously (37). Thus, the reversal of the bradycardia in hypothyroidism following T_3 treatment may represent T_3 action in brain, as opposed to direct T_3 action in the heart. Therefore, those functions in peripheral organs that are regulated by the brain

would be expected to show stereospecific differences in the biological potency of D- vs L-T_3, since these two isomers are transported through the BBB at markedly different rates (36).

RECEPTOR-MEDIATED TRANSPORT

Using isolated brain capillaries as an in vitro model system of the BBB has led to the discovery of many different BBB peptide receptors (Table 2). The function of the BBB peptide receptors may be to (*a*) act as transport systems (38); (*b*) inititate signal transduction pathways within the brain capillary endothelium, e.g. activation of adenyl cyclase or guanyl cyclase; or (*c*) alter BBB permeability to circulating nutrients, water, or plasma proteins.

Recent in vivo experiments show that the BBB insulin and transferrin receptors mediate the net transport of the circulating peptide into brain interstitial space (39, 40). This process is receptor-mediated transcytosis. It is believed to involve three sequential steps (38): (*a*) receptor-mediated endocytosis at the blood side of the BBB; (*b*) diffusion of the peptide or peptide-receptor complex through the endothelial cytoplasm; and (*c*) receptor-mediated exocytosis of the peptide at the brain side of the BBB into brain interstitial space. This transport process explains the origin of insulin in brain (41), since the de novo synthesis of insulin does not occur in brain in vivo (42). The transcytosis of transferrin through the BBB undoubtedly accounts for the distribution of circulating iron into brain (43), which it needs on a minute-to-minute basis to sustain intermediary metabolism. Transferrin-bound aluminum may also explain the deposition of this mineral in brain interstitial space, particularly in Alzheimer's disease, where aluminum accumulates in the core of the neuritic plaque (44).

Table 2 Blood-brain barrier peptide receptors[a]

Species	Peptide	K_D (nM)	R_O (pmol/mg_p)
Human	insulin	1.2 ± 0.5	0.17 ± 0.08
	IGF-1[b]	2.1 ± 0.4	0.17 ± 0.02
	IGF-2	1.1 ± 0.1	0.21 ± 0.01
	transferrin	5.6 ± 1.4	0.10 ± 0.02
Bovine	insulin	0.44	0.18
	IGF-1	2.0	1.7
	IGF-2	1.8	1.0
	atriopeptin	0.11	0.058
Canine	angiotensin II	1.1	0.022

[a] From (43, 83–88).
[b] IGF = insulin-like growth factor.

Another important component of the neuritic plaque in Alzheimer's disease is the amyloid peptide, called the A_4 peptide or β-peptide (45, 46), which accumulates around cortical microvessels in Alzheimer's disease (47). The A_4 peptide arises from a high-molecular-weight precursor normally found in brain and peripheral organs (48, 49). Using a radioimmunoassay and an antiserum directed against a synthetic fragment of the A_4 peptide, recent studies indicate the A_4 peptide precursor may be related to a circulating immunoglobulin G (50). Moreover, the CSF concentration of the high-molecular-weight immunoreactive A_4 peptide precursor is tenfold higher than the usual concentration of plasma proteins (50). This suggests that specific plasma proteins, such as the putative precursor of the A_4 amyloid peptide, may be selectively transported through the BBB, possibly via receptor-mediated transcytosis systems analagous to those for insulin or transferrin (38).

Some of the BBB peptide receptors may not mediate the net transport of the peptide into brain interstitial space, but may function to entrap the circulating peptide and initiate single transduction pathways within the brain capillary endothelial cytoplasm. For example, parathyroid hormone or vasoactive intestinal peptide activates brain capillary adenyl cyclase (51), and atriopeptin increases brain capillary guanyl cyclase (52). Activation of cyclases may lead to changes in protein phosphorylation or dephosphorylation in the brain capillary, since these pathways are nearly as active in the brain capillary as in brain synaptosomes (53). The net effect of peptide-mediated changes in signal transduction pathways in the brain capillary may be an alteration in the permeability properties to circulating nutrients, water, or plasma proteins. For example, angiotensin II and vasopressin alter BBB transport of water (54, 55).

Another important pathway of interaction between peptides and the brain capillary is enzymatic degradation of peptides. Brain capillaries contain abundant quantities of aminopeptidase, which is active on enkephalins or [Tyr^1]somatostatin (56, 57), and peptidyl dipeptidases such as angiotensin-converting enzyme (58, 59). The latter enzyme converts angiotensin I to angiotensin II, which is a potent vasoconstrictor, and inactivates bradykinin, which is a potent vasodilator. Therefore, drugs such as captopril, which inhibit angiotensin-converting enzyme, may have a profound effect on vasoactive peptide metabolism at the brain microcirculation.

PLASMA PROTEIN–MEDIATED TRANSPORT

Steroid hormones and free fatty acids are highly lipid soluble and are transported through the BBB by lipid-mediated transport (60). However, these substances are avidly bound by circulating plasma proteins, such as albumin or specific globulins. Previously, only the free fraction was thought to be

available for transport into brain or into other organs in vivo. However, recent studies show that the bound hormone or free fatty acid is operationally available for transport through the BBB withlout significant exodus of the plasma protein per se from the brain microcirculation (60, 61). This fact arises from enhanced rates of ligand dissociation from ciculating plasma proteins such as albumin, owing to putative conformational changes about the ligand binding site that may follow from transient interactions between the plasma protein and the surface of the brain microcirculation (60). Using a tracer kinetic model, the dissociation constant (K_D^a) may be quantitated (Table 3). In the case of albumin-bound lipophilic amines, such as propanolol or bupivacaine, the dissociation constant within the brain microcirculation is not statistically different from the dissociation constant measured in vitro (K_D). However, the K_D^a in vivo for the binding of these two drugs to α_1-acid glycoprotein (also called orosomucoid) is severalfold greater than the corresponding in vitro K_D (Table 3). In addition, the K_D^a in vivo for the binding of a number of steroid hormones, tryptophan, or T_3 to albumin in the brain microcirculation is larger than the corresponding in vitro value (Table 3). The markedly increased rates of ligand dissociation in vivo probably result from relatively minor conformational changes that take place wtihin the brain microcirculation. For example, a recent study using X-ray diffraction shows that the in vitro K_D of ligand binding to a protein is increased two-to-three log orders of magnitude by the removal of a single hydrogen bond from the ligand binding site (62). Since virtually all proteins mediate their function through conformational changes, plasma proteins may actually deliver ligands to tissues via endothelial-induced conformational changes of the ligand binding within the microcirculation.

Table 3 Comparison of bovine albumin and human α_1-acid glycoprotein (AAG) dissociation constant in vivo in brain capillary (K_D^a) and in vitro (K_D)[a]

Plasma protein	Ligand	K_D (μM) (in vitro)	K_D^a (μM) (in brain capillary)
Bovine albumin	testosterone	53 ± 1	2,520 ± 710
	tryptophan	130 ± 30	1,670 ± 110
	corticosterone	260 ± 10	1,330 ± 90
	dihydrotestosterone	53 ± 6	830 ± 140
	estradiol	23 ± 1	710 ± 100
	propranolol	290 ± 30	220 ± 40
	bupivacaine	141 ± 10	211 ± 107
	T_3[b]	4.7 ± 0.1	46 ± 4
Human AAG	propranolol	3.3 ± 0.1	19 ± 4
	bupivacaine	6.5 ± 0.5	17 ± 4

[a] From (60, 89, 90).
[b] T_3 = triiodothyronine

The fact that the bound drug is, in many cases, operationally available for transport through the BBB means that present pharmacokinetic models that assume that only free drug is transported must be reevaluated. The capillary-exchangeable hormone should, when possible, be measured with in vivo techniques, since in vitro measurements of free hormone may greatly underestimate the concentration of capillary exchangeable hormone or the concentration of cellular free hormone (60). The latter is believed to interact with drug receptors in brain, and drug receptor occupancy likely determines the organ-specific pharmacodynamics.

DRUG DELIVERY

The various strategies for drug delivery through the BBB are shown in Table 4 and may be categorized as (*a*) invasive; (*b*) pharmacologic-based strategies; and (*c*) physiologic-based strategies. The intraventricular administration of drugs such as bethanechol, which is a cholinomimetic, has been used in the treatment of Alzheimer's disese (63). The intraventricular infusion of the drug in humans is made possible with an implantable pump. However, other studies show that the intraventricular administration of drug results in primarily bathing the surface of the brain only, since the efflux of the drug out of the ventricular compartment into the superior sagittal sinus is much faster than diffusion of drug into brain parenchyma (64). Therefore, while the intraventricular administration of drug may be useful for diseases that have a predilection for the meninges, e.g. leukemic infiltration, the ventricular approach, in addition to being invasive, may not be useful therapy for delivery of drug well into the brain parenchyma. An interesting alternative approach to the placement of an intraventricular cannula is the intranasal administration of lipid-soluble substances. One study shows that the nasal administration of the steroid hormone progesterone, which is highly lipid soluble, results in a higher concentration in cerebrospinal fluid, as compared to the corresponding serum concentration (65). This finding suggests that the

Table 4 Strategies for drug delivery through the blood-brain barrier [a]

1.	Invasive
	intracarotid infusion of hypertonic media
	intraventricular infusion
2.	Pharmacologic
	liposomes
	lipid-soluble pro-drugs
3.	Physiologic
	chimeric nutrients
	chimeric peptides

[a] From (9, 38).

progesterone has direct access to the CSF compartment following nasal administration. In fact, recent studies show that the submucous space of the nose is in direct contact with the subarachnoid space of olfactory lobes (66). However, it is unlikely that water-soluble substances such as peptides will gain direct access to the CSF following intranasal administration, since these substances may not diffuse through the barriers separating the nasal submucous spaces and the olfactory lobe subarachnoid space.

Pharmacologic-based strategies often involve the use of liposomes (67). However, these substances are only taken up by cells lining the reticuloendothelial system (67), and do not appear to be useful for delivery of drugs through the BBB. Although liposomes are highly lipid soluble, they are apparently too large to pass through the BBB via lipid-mediated transport. An example of the size exclusion of high-molecular-weight lipid-soluble substances is the peptide cyclosporin. This cyclic undecapeptide of fungal origin is highly lipid soluble with a 1-octanol/Ringer's partition coefficient of 991 ± 55 (68). However, the BBB transport of ^{3}H-cyclosporin is barely greater than the transport of a vascular space marker such as ^{3}H-inulin (68).

A promising pharmacologic-based strategy for drug delivery through the BBB is drug latentiation or formation of lipid-soluble pro-drugs from water soluble drugs (69). The most highly developed strategy in this regard is the coupling of water-soluble drugs to a pyridine nucleus (70). This approach has two advantages. First, it increases the lipid solubility of the drug, owing the the highly lipid-soluble nature of the pyridine carrier, and second, brain oxidative enzymes convert the pyridine base to a quaternary pyridinium salt, which effectively entraps the drug in the brain cellular compartment (70). This delivery system is similar in form to the MPTP-MPP^{+} interconversion in experimental Parkinson's disease; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a toxic contaminant of synthetic heroin and is converted to the pyridinium salt (MPP^{+}) by monoamine oxidase type B in brain (71).

Another application of pharmacologic-based BBB delivery systems is the synthesis of lipid-soluble technetium analogues, such as Tc^{99m}-labeled 1,2-dithia-5,8-diazacyclobecane (BAT) chelate (72). Water-soluble technetium derivatives are used in nuclear medicine for brain scanning to reveal breakdown of the BBB, since the water-soluble technetium derivatives do not cross the barrier. However, lipid-soluble technetium derivatives could be used in neuroimaging procedures to measure regional changes in cerebral blood flow that may parallel regional differences in brain function. With the pyridine-pyridinium delivery system described above, the best imaging agent, however, is a lipid-soluble technetium agent that is entrapped in brain so that rapid washout of the substance is prevented. The placement of a three- to four-carbon primary or secondary amine tail on the lipid soluble technetium agent may enhance the sequestration of the molecule in brain, similar to that found for other lipophilic amines such as propranolol or lidocaine (73).

The physiologic-based strategies include the development of chimeric nutrients or chimeric peptides (9, 38). These are pro-drugs that are transported through the BBB owing to their affinity for one of the specific carrier-mediated transport systems for ciculating nutrients or receptor-mediated transcytosis systems for circulating peptides. For example, drugs that may be thought of as chimeric nutrients include L-DOPA, α-methyl-DOPA, α-methylparatyrosine, or phenylalanine mustard (melphalan). These agents all cross the BBB on the neutral amino acid transport system (74–76). Because of this, the pharmacodynamics of the drug may be altered by nutritional factors. The insulin secretion following carbohydrate administration results in hypoaminoacidemia and desaturation of the neutral amino acid transport system (77). This allows for increased α-methyl-DOPA transport through the BBB and increased drug efficacy in lowering blood pressure (75). Conversely, the administration of a high protein meal results in a hyperaminoacidemia that inhibits the BBB transport of L-DOPA and decreases the efficacy of this drug in the treatment of Parkinson's disease (74). Although most chemotherapeutic agents do not cross the BBB, melphalan is one example of a polar oncologic agent that does cross, owing to it's affinity for the BBB neutral amino acid transport system (76). These examples illustrate (*a*) that amines can be made to cross the BBB by converting the amine to an α-amino acid; or (*b*) that the placement of active drug moieties, e.g. alkylating groups, on an aromatic amino acid nucleus can result in the formation of a new drug that is transported through the BBB.

The synthesis of chimeric peptides is a new approach to the delivery of nontransportable peptides through the BBB (9, 38, 78). Peptides may be classified broadly as either being transportable, i.e. having an affinity for a BBB transcytosis system (e.g. insulin, transferrin, or insulinlike growth factors), or nontransportable, i.e. having little or no affinity for a specific BBB transport system (e.g. enkephalins, β-endorphin, and numerous other peptides) (38). The chimeric peptide is formed when a transportable peptide is covalently coupled to a nontransportable peptide, preferably using a cross-linking reagent that can be cleaved once in brain. For example, disulfide-based cross-linking reagents used to prepare a β-endorphin chimeric peptide (78) are stable in plasma, but should be cleavable in brain by thiol reductases (79).

Recent studies examined this approach by coupling β-endorphin (a nontransportable peptide) to cationized albumin (a transportable peptide) using the cross-linking reagent *N*-succinimidyl 3-(2-pyridyldithio) proprionate (SPDP) (78). The cationization of albumin, which normally has an isoelectric point of approximately 4, to a derivative that is highly positively charged with an isoelectric point of 8.5–9 causes this plasma protein to be rapidly transported into cerebrospinal fluid (80), and across brain capillaries

via a process of absorptive-mediated transcytosis (78). Using isolated brain capillaries as an in vitro model system of the BBB, ^{3}H-cationized albumin (pI = 8.5–9) is rapidly endocytosed by an active receptor-mediated system (K_D = 0.8 μM, R_O = 79 pmole/mg protein; see Table 2 for comparison). The uptake of ^{3}H-cationized albumin is competitively inhibited by other polycationic substances like protamine or polylysine (with a $K_i \sim 3$ μg/ml) (78). Moreover, the β-endorphin-SPDP-cationized albumin chimeric peptide is also rapidly taken up and endocytosed by isolated bovine brain capillaries via a process that is saturated by unlabeled cationized albumin, but not by unconjugated β-endorphin or native albumin (pI = 4) (78). A major challenge in the understanding of cell biology of peptide delivery through the BBB is to identify the endothelial subcellular organelles involved in peptide trafficking from the blood pole of the capillary endothelium to the brain side.

SUMMARY

In summary, recent studies over the last ten years have concentrated on what the blood-brain barrier does rather than what it is. This focus has changed the concept of this important membrane from a passive, relatively immutable structure to a dynamic interface between blood and brain. Further understanding of the molecular cell physiology of the brain capillary endothelium will undoubtedly lead to new insights into both drug action at the BBB and drug delivery through this barrier.

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Literature Cited

1. Pardridge, W. M. 1987. The gate keeper: How molecules are screened for admission to the brain. *The Sciences* 27:50–55
2. Brightman, M. W., Reese, T. S., Feder, N. 1970. Assessment with the electron-microscope of the permeability to peroxidase of cerebral endothelium and epithelium in mice and sharks. In *Capillary Permeability* (Alfred Benzon Symposium II) pp. 468–86. Copenhagen: Munksgaard
3. Mollgard, K., Saunders, N. R. 1975. Complex tight junctions of epithelial and of endothelial cells in early foetal brain *J. Neurocytol.* 4:453–68
4. Brightman, M. W. 1977. Morphology of blood-brain interfaces. *Exp. Eye Res.* 25:1–25 (Suppl.)
5. Pardridge, W. M. 1983. Neuropeptides and the blood-brain barrier. *Ann. Rev. Physiol.* 45:73–82
6. Oldendorf, W. H. 1971. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* 221:1629–39
7. Hawkins, R. A. 1986. Transport of essential nutrients across the blood-brain barrier of individual structures. *Fed. Proc.* 45:2055–59
8. Goldstein, G. W., Betz, A. L. 1983. Recent advances in understanding brain

capillary function. *Ann. Neurol.* 14: 389–95
9. Pardridge, W. M. 1985. Strategies for drug delivery through the blood-brain barrier. In *Directed Drug Delivery: A Multidisciplinary Problem,* ed. R. T. Borchardt, A. J. Repta, V. J. Stella, pp. 83–96 New Jersey: Humana
10. Stewart, P. A., Wiley, M. J. 1981. Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: A study using quail-chick transplantation chimera. *Dev. Bio.* 84:183–92
11. Janzer, R. C., Raff, M. C. 1987. Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 325: 253–57
12. Cancilla, P. A., DeBault, L. E. 1983. Neutral amino acid transport properties of cerebral endothelial cells in vitro. *J. Neuropathol. Exp. Neurol.* 42:191–99
13. Bowman, P. D., Ennis, S. R., Rarey, K. E., Betz, A. L., Goldstein, G. W. 1983. Brain microvessel endothelial cells in tissue culture: A model for study of blood-brain barrier permeability. *Ann. Neurol.* 14:296–302
14. Sage, M. R. 1982. Blood-brain barrier: Phenomenon of increasing importance to the imaging clinician. *Am. J. Roentgenol.* 138:887–98
15. Crone, C. 1965. Facilitated transfer of glucose from blood into brain tissue. *J. Physiol. London* 181:103–13
16. Gjedde, A. 1983. Modulation of substrate transport to the brain. *Acta Neurol. Scand.* 67:3–25
17. Dick, A. P., Harik, S. J., Klip, A., Walker, D. M. 1984. Identification and characterization of the glucose transporter of the blood-brain barrier by cytochalasin B binding and immunological reactivity. *Proc. Natl. Acad. Sci USA* 81:7233–37
18. Baldwin, S. A., Cairns, M. T., Gardiner, R. M., Ruggier, R. 1985. A D-glucose-sensitive cytochalasin B binding component of cerebral microvessels. *J. Neurochem.* 45:650–52
19. Mueckler, M. C., Caruso, C., Baldwin, S. A, Panico, M., Blench, I., et al. 1985. Sequence and structure of a human glucose transporter. *Science* 229: 941–45
20. Birnbaum, M. J., Haspel, H. C., Rosen, O. M. 1986. Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. *Proc. Natl. Acad. Sci. USA* 83:5784–88
21. Flier, J. S., Mueckler, M., McCall, A. L., Lodish, H. F. 1987. Distribution of glucose transporter messenger RNA transcripts in tissues of rat and man. *J. Clin. Invest.* 79:657–61
22. Dick, A.P.K., Harik, S. I. 1986. Distribution of the glucose transporter in the mammalian brain. *J. Neurochem.* 46: 1406–11
23. Gjedde, A., Crone, C. 1981. Blood-brain glucose transfer: Repression in chronic hyperglycemia. *Science* 214:456–57
24. McCall, A. L., Millington, W. R., Wurtman, R J. 1982. Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. *Proc. Natl. Acad. Sci. USA* 79:5406–10
25. McCall, A. L., Fixman, L. B., Tornheim, K., Check, W., Ruderman, N. B. 1986. Chronic hypoglycemia increases brain glucose transport. *Am. J. Physiol.* 251:E442–47
26. Oldendorf, W. H. 1970. Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res.* 24:372–76
27. Pardridge, W. M., Landaw, E. M., Miller, L. P., Braun, L. D., Oldendorf, W. H. 1985. Carotid artery injection technique: Bounds for bolus mixing by plasma and by brain. *J. Cereb. Blood Flow Metab.,* 5:576–83
28. Smith, Q. R., Takasato, Y, Sweeney, D. J., Rapoport, S. I. 1985. Regional cerebrovascular transport of leucine as measured by the in situ brain perfusion technique. *J. Cereb. Blood Flow Metab.* 5:300–11
29. Choi, T., Pardridge, W. M. 1986. Phenylalanine transport at the human blood-brain barrier. Studies in isolated human brain capillaries. *J. Biol. Chem.* 261:6536–41
30. Binek-Singer, P., Johnson, T. C. 1982. The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids. *Biochem. J.* 206:407–14
31. Pardridge, W. M. 1986. Potential effects of the dipeptide sweetener aspartame on the brain. In *Nutrition and the Brain,* ed. R. J. Wurtman, J. J. Wurtman, 7:199–241. New York: Raven
32. Filer, L. J. Jr., Stegink, L. D. 1988. Effect of aspartame on plasma phenylalanine concentration in humans. In *Dietary Phenylalanine and Brain Function,* ed. R. J. Wurtman, E. Ritter-Walker. Boston: Birkhauser. In press
33. Wurtman, R. J., Fernstrom, J. D. 1975. Control of brain monoamine synthesis by diet and plasma amino acids. *Am. J. Clin. Nutr.* 28:538–647
34. Boyd, G. S., Oliver, M. F. 1960. Vari-

ous effects of thyroxine analogues on the heart and serum cholesterol in the rat. *J. Endocrinol.* 21:25–32
35. DeGroot, L. J., Torresani, J. 1975. Triiodothyronine binding to isolated liver cell nuclei. *Endocrinology* 96: 357–63
36. Terasaki, T., Pardridge, W. M. 1987. Stereospecificity of triiodothyronine transport into brain, liver, and salivary gland: Role of carrier- and plasma protein–mediated transport. *Endocrinology* 121:1185–1191
37. Goldman, M., Dratman, M. B., Crutchfield, F. L., Jennings, A. S., Maruniak, J. A., Gibbons, R. 1985. Intrathecal triiodothyronine administration causes greater heart rate stimulation in hypothyroid rats than intravenously delivered hormone. *J. Clin. Invest.* 76: 1622–27
38. Pardridge, W. M. 1986. Receptor-mediated peptide transport through the blood-brain barrier. *Endocr. Rev.* 7: 314–30
39. Duffy, K. R., Pardridge, W. M. 1987. Blood-brain barrier transcytosis of insulin in developing rabbits. *Brain Res.* 420:32–38
40. Fishman, J., Rubin, J. B., Handarhan, J. V., Connor, J., Fine, R. E. 1987. Receptor-mediated transcytosis of transferrin across the blood-brain barrier. *J. Neurosci. Res.* In press
41. Havrankova, J., Roth, J. 1979. Concentrations of isulin and of insulin receptors in the brain are independent of peripheral insulin levels. *J. Clin. Invest.* 64:636–42
42. Giddings, S. J., Chirgwin, J., Permutt, M. A. 1985. Evaluation of rat messenger RNA in pancreatic and extrapancreatic tissues. *Diabetologica* 28:343–49
43. Pardridge, W. M., Eisenberg, J., Yang, J. 1987. Human blood-brain barrier transferrin receptor. *Metabolism* 36: 892–895
44. Perl, D. P. 1983. Aluminum and Alzheimer's disease: Intraneuronal X-ray spectometry studies. In *Branbury Report 15: Biological Aspects of Alzheimer's Disease,* ed. R. Katzman, Cold Springs Harbor Lab.
45. Glenner, G. G., Wong, C. W. 1984. Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* 122: 1131–35
46. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., Beyreuther, K. 1985. Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. *Proc. Natl Acad. Sci. USA* 82:4245–49
47. Pardridge, W. M., Vinters, H. V., Yang, J., Eisenberg, J., Choi, T., et al. 1987. Amyloid angiopathy of Alzheimer's disease: Amino acid composition and partial sequence of a 4200 dalton peptide isolated from cortical microvessels. *J. Neurochem.* 49:1394–1401
48. Goldgaber, D., Lerman, I., McBride, O. W., Saffiotti, U., Fajdusek, D. C. 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 235:877–80
49. Tanzi, R. E., Gusell, J. F., Watkins, P. C., Bruns, G.A.P., St. George-Hyslop, P., et al. 1987. Amyloid β-protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 235:880–84
50. Pardridge, W. M., Vinters, H. V., Miller, B. L., Tourtellotte, W. W., Eisenberg, J., Yang, J. 1987. High molecular weight Alzheimer's disease amyloid peptide immunoreactivity in human serum and CSF is an immunoglobulin G. *Biochem. Biophys. Res. Commun.* 145: 241–248
51. Huang, M., Rorstad, O. P. 1984. Cerebral vascular adenylate cyclase: Evidence for coupling to receptors for vasoactive intestinal peptide and parathyroid hormone. *J. Neurochem.* 43: 849–54
52. Steardo, L., Nathanson, J. A. 1987. Brain barrier tissues: End organs for atriopeptins. *Science* 235:470–73
53. Pardridge, W. M., Yang, J., Eisenberg, J. 1985. Blood-brain barrier protein phosphorylation and dephosphorylation. *J. Neurochem.* 45:1141–47
54. Grubb, R. L. Jr., Raichle, M. E. 1981. Intraventricular angiotensin II increases brain permeability. *Brain Res.* 210:426–30
55. Raichle, M. E., Grubb, R. L. Jr. 1978. Regulation of brain water permeability by centrally-released vasopressin. *Brain Res.* 143:191–94
56. Pardridge, W.M., Mietus, L. J. 1981. Enkephalin and blood-brain barrier: Studies of binding and degradation in isolated brain microvessels. *Endocrinology* 109:1138–43
57. Pardridge, W. M., Eisenberg, J., Yamada, T. 1985. Rapid sequestration and degradation of somatostatin analogues by isolated brain microvessels. *J. Neurochem.* 44:1178–84
58. Brecher, P., Tercyak, A., Gavras, H., Chobanian, A. V. 1978. Peptidyl di-

peptidase in rabbit brain microvessels. *Biochim. Biophys. Acta* 526:537–43
59. Kobayashi, H., Wada, A., Izumi, F., Take, K., Magnoni, M. S. 1985. Low activity of angiotensin-converting enzyme in cerebral microvessels of young spontaneously hypertensive rats. *J. Neurochem.* 44:1318–20
60. Pardridge, W. M. 1987. Plasma protein-mediated transport of steroid and thyroid hormones. *Am. J. Physiol.* 252:E157–64
61. Pardridge, W. M., Eisenberg, J., Cefalu, W. T. 1985. Absence of albumin receptor on brain capillaries in vivo or in vitro. *Am. J. Physiol.* 249:E264–67
62. Bartlett, P. A., Marlowe, C. K. 1987. Evaluation of intrinsic binding energy from a hydrogen bonding group in an enzyme inhibitor. *Science* 235:569–71
63. Harbaugh, R. E., Roberts, D. W., Coombs, D. W., Saunders, R. L., Reeder, T. M. 1984. Preliminary report: Intracranial cholinergic drug infusion in patients with Alzheimer's disease. *Neurosurgery* 15:514–18
64. Covell, D. G., Narang, P. K., Poplack, D. G. 1985. Kinetic model for disposition of 6-mercaptopurine in monkey plasma and cerebrospinal fluid. *Am. J. Physiol.* 248:R147–56
65. Kumar, T. C., David, A., Sankaranaravanan, G.F.X., Puri, V., Sundram, P. 1982. Pharmacokinetics of progesterone after its administration to ovariectomized Rhesus monkeys by injection, infusion, or nasal spraying. *Proc. Natl. Acad. Sci. USA* 79:4185–89
66. Bradbury, M.W.B., Cserr, H. F., Westrop, R. J. 1981. Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. *Am. J. Physiol.* 240:F329–36
67. Patel, J. M. 1984. Liposomes: Bags of challenge *Biochem. Soc. Trans.* 12:333–35
68. Cefalu, W. T., Pardridge, W. M. 1985. Restrictive transport of a lipid-soluble peptide (cyclosporin) through the blood-brain barrier. *J. Neurochem.* 45:1954–56
69. Oldendorf, W. H. 1974. Blood-brain barrier permeability to drugs. *Ann. Rev. Pharmacol.* 14:239–48
70. Bodor, N., Brewster, M. E. 1983. Problems of delivery of drugs to the brain *Pharmac. Ther.* 19:337–86
71. Lewin, R. 1984. Brain enzyme is the target of drug toxin. *Science* 225:1460–62
72. Kung, H. F., Molnar, M., Billings, J., Wicks, R., Blau, M. 1984. Synthesis and biodistribution of neutral lipid-soluble Tc-99m complexes that cross the blood-brain barrier. *J. Nucl. Med.* 25: 326–32
73. Pardridge, W. M., Sakiyama, R., Fierer, G. 1984. Blood-brain barrier transport and brain sequestration of propranolol and lidocaine. *Am. J. Physiol.* 247:R582–88
74. Nutt, J. G., Woodward, W. R., Hammerstad, J. P., Carter, J. H., Anderson, J. L. 1984. The "on-off" phenomenon in Parkinson's disease: Relation to levodopa absorption and transport. *N. Engl. J. Med.* 310:483–88
75. Markovitz, D. C., Fernstrom, J. D. 1977. Diet and uptake of aldomet by the brain: Competition with natural large neutral amino acids. *Science* 197:1014–15
76. Greig, N. H., Momma, S., Sweeney, D. J., Smith, Q. R., Rapoport, S. I. 1987. Facilitated transport of melphalan at the rat blood-brain barrier by the large neutral amino acid carrier system. *Cancer Res.* 47:1571–76
77. Fernstrom, J. D., Wurtman, R. J. 1972. Brain serotonin content: Physiological regulation by plasma neutral amino acids *Science* 178:414–16
78. Kumagai, A. K., Eisenberg, J., Pardridge, W. M. 1987. Absorptive-mediated endocytosis of cationized albumin and a β-endorphin-cationized albumin chimeric peptides by isolated brain capillaries. Model system of blood-brain barrier transport. *J. Biol. Chem.* 262:15214–19
79. Letvin, N. L., Goldmacher, V. S., Ritz, J., Yetz, J. M., Schlossman, S. F., Lambert, J. M. 1986. In vivo administration of lymphocyte-specific monoclonal antibodies in nonhuman primates. *J. Clin. Invest.* 77:977–84
80. Griffin, D. E., Giffels, J. 1982. Study of protein characteristics that influence entry into the cerebrospinal fluid of normal mice and mice with encephalitis. *J. Clin. Invest.* 70:289–95
81. Pardridge, W. M. 1983. Brain metabolism: A perspective from the blood-brain barrier. *Physiol. Rev.* 63:1481–535
82. Pardridge, W. M. 1979. Carrier-mediated transport of thyroid hormones through the blood-brain barrier. Primary role of albumin-bound hormone. *Endocrinology* 105:605–12
83. Pardridge, W. M., Eisenberg, J., Yamada, T. 1985. Human blood-brain barrier insulin receptor. *J. Neurochem.* 44: 1771–78
84. Duffy, K. R., Pardridge, W. M., Rosenfeld, R. G. 1988. Human blood-brain barrier insulin-like growth factor receptor. *Metabolism.* In press

85. Frank, H. J. L., Pardridge, W. M. 1981. A direct in vitro demonstration of insulin binding to isolated brain microvessels. *Diabetes* 30:757–61
86. Frank, H.J.L., Pardridge, W. M., Morris, W. L., Rosenfeld, R. G., Choi, T. B. 1986. Binding and internalization of insulin and insulin-like growth factors by isolated brain microvessels. *Diabetes* 35:654–61
87. Chabrier, P. E., Roubert, P., Braquet, P. 1987. Specific binding of atrial natriuretic factor in brain microvessels. *Proc. Natl. Acad. Sci. USA* 84:2078–81
88. Speth, R. C., Harik, S. I. 1985. Angiotensin II receptor binding sites in brain microvessels. *Proc. Natl. Acad. Sci. USA* 82:6340–43
89. Pardridge, W. M., Landaw, E. 1984. Tracer kinetic model of blood-brain barrier transport of plasma protein-bound ligands. Empiric testing of the free hormone hypothesis. *J. Clin. Invest.* 74: 745–52
90. Terasaki, T., Pardridge, W. M., Denson, D. D 1986. Differential effects of plasma protein binding of bupivacaine on its in vivo transfer into the brain and salivary gland of rats. *J. Pharmacol. Exp. Ther.* 239:724–29