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Delivery of therapeutic agents to the central nervous system: the problems and the possibilities

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Abstract

The presence of a blood-brain barrier (BBB) and a blood-cerebrospinal fluid barrier presents a huge challenge for effective delivery of therapeutics to the central nervous system (CNS). Many potential drugs, which are effective at their site of action, have failed and have been discarded during their development for clinical use due to a failure to deliver them in sufficient quantity to the CNS. In consequence, many diseases of the CNS are undertreated. In recent years, it has become clear that the blood-CNS barriers are not only anatomical barriers to the free movement of solutes between blood and brain but also transport and metabolic barriers. The cell association, sometimes called the neurovascular unit, constitutes the BBB and is now appreciated to be a complex group of interacting cells, which in combination induce the formation of a BBB. The various strategies available and under development for enhancing drug delivery to the CNS are reviewed.

Keywords: Blood-brain barrier; CNS drug delivery; Drug targeting to the CNS; Cerebral capillary endothelium; Choroid plexus; Circumventricular organs

Abbreviations: Å, angstrom unit; ABC, ATP-binding cassette; AMT, absorptive-mediated transcytosis; Apo-E, apolipoprotein E; ATP, adenosine triphosphate; AUC, area under the curve; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BCSFB, blood-cerebrospinal fluid barrier; BDNF, brain-derived neurotrophic factor; BUI, brain uptake index; CDS, chemical delivery system; CNS, central nervous system; CSF, cerebrospinal fluid; CVO, circumventricular organ; D,L-NAM, D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid; GFAP, glial fibrillary acidic protein; GLUT1, glucose uptake transporter 1; HIV, human immunodeficiency virus; ISF, interstitial fluid; kD, kilo Daltons; LDL, low-density lipoprotein; L-DOPA, dihydroxyphenylalanine; LNAA, large neutral amino acid transporter (L-system); mAb, monoclonal antibody; MRP, multidrug resistance protein; MTX, methotrexate; NGF, nerve growth factor; OX26, monoclonal antibody; PBCA, poly(butyl)cyanoacrylate; PEG, polyethylene glycol; Pgp, P-glycoprotein (permeability glycoprotein); pH, reciprocal of logarithm hydrogen ion concentration; RMT, receptor-mediated transcytosis; SAR, structure-activity relationship; Syn B1, Protegrin-derived pegelin protein; TAT, transactivating-transduction protein; ZO, zona occludens.

Contents

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1. Introduction

All animals with a complex nervous system require a blood-brain barrier (BBB). The BBB allows the creation of a unique extracellular fluid environment within the central nervous system (CNS) whose composition can, as a consequence, be precisely controlled. The extracellular fluid compartments of the CNS comprise the brain and spinal cord parenchymal interstitial fluid (ISF) and the cerebrospinal fluid (CSF), contained within the ventricles of the brain and the cerebral and spinal subarachnoid spaces. The structural BBB is created by the cerebral endothelial cells forming the capillaries of the brain and spinal cord (Fig. 1). The endothelial cells at their adjacent margins form tight junctions (zona occludens [ZO]; Brightman & Reese, 1969), produced by the interaction of several transmembrane proteins that project into and seal the paracellular pathway. The interaction of these junctional proteins, particularly occludin and claudin, is complex and effectively blocks an aqueous route of free diffusion for polar solutes from blood along these potential paracellular pathways and thus denies these solutes free access to brain interstitial (extracellular) fluid. The molecular structure and function of the BBB junctional proteins is beyond the scope of this review, but several recent reviews exist (Morita et al., 1999; Kniesel & Wolburg, 2000; Wolburg et al., 2001; Bauer et al., 2004; Hamm et al., 2004). The impediment to free diffusion is, of course, bidirectional and therefore does not allow a free diffusional movement of polar solutes out of the CNS. Because the tight junctions effectively seal off the brain to polar solutes, the endothelial cells are required to maintain a high level of expression of transport proteins for essential polar metabolites such as glucose and amino acids to facilitate their entry into brain (Begley & Brightman, 2003). Thus, the tight junctions between the endothelial cells form an efficient gate in the paracellular pathway, preventing the diffusional entry of polar solutes to the brain via this route.

Electron microscopic studies of the BBB suggest a lower incidence of observable endocytic profiles in these endothelial cells compared with peripheral endothelial cells. However, transcytosis involving vesicular transport across the BBB is a significant factor in the BBB transport of many macromolecules, such as peptides and proteins, and both receptor-mediated transcytosis (RMT) and nonspecific absorptive-mediated transcytosis (AMT) pathways exist across the cerebral endothelium (Begley & Brightman, 2003).

The endothelial cells forming the BBB also exhibit a polarized expression of transport proteins in the luminal and abluminal membranes of the endothelial cells, with some transporters expressed exclusively in one of these interfacial membranes and some in the other, whereas some are inserted into both membranes (Betz et al., 1980; Begley, 1996; Mertsch & Maas, 2002; Begley & Brightman, 2003). As some transporters are unidirectional and some bidirectional in

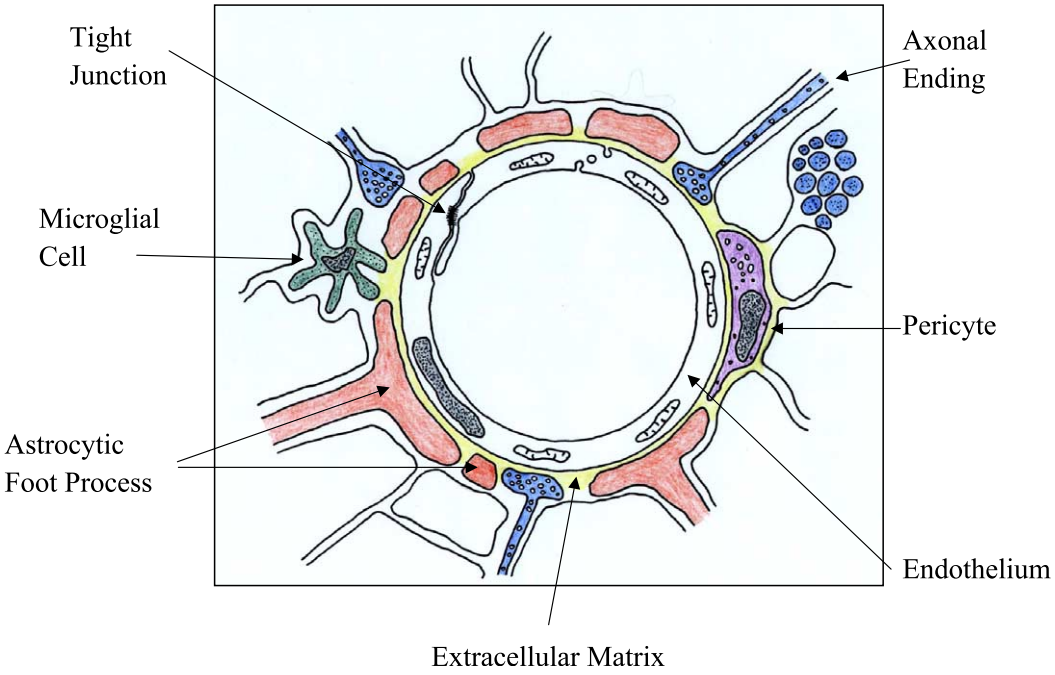


Fig. 1. Schematic diagram of the neurovascular unit/cell association forming the BBB. The cerebral endothelial cells form tight junctions at their margins where they meet, which completely seal the aqueous paracellular pathways between the cells. Pericytes are distributed discontinuously along the length of the cerebral capillaries and partially surround the endothelium. Both the cerebral endothelial cells and the pericytes are surrounded by, and contribute to, the same extracellular matrix rich in laminins 8 and 10. Foot processes from astrocytes form a network fully surrounding the capillaries. Axons from neurons also abutt closely against the endothelial cells and contain vasoactive neurotransmitters and peptides. Microglia (perivascular macrophages) are the resident immunocompetent cells of the brain and are derived from systemic circulating monocytes and macrophages. The movement of solutes across the BBB is either passive, driven by a diffusion gradient with more lipid-soluble substances having a greater BBB penetration or may be facilitated by passive or active transporters, inserted into the luminal or abluminal membranes of the endothelial cells. Efflux transporters may limit the CNS penetration of several solutes.

their transport of solutes across the cell membrane, this polarization means that some solutes can be preferentially transported into the brain and some out of the brain and that the transport of some solutes can be facilitated in either direction depending on whether the concentration gradient across the BBB is directed into, or out of, the CNS. This latter aspect can become important for some drugs with affinity for BBB transporters, when after systemic administration the pharmacokinetic profile can cause the concentration gradient to reverse across the BBB. It is thought that the formation of tight junctions between the endothelial cells may also act as a fence in the cell membrane preventing both transport proteins and lipid rafts in the membrane from exchanging between the luminal and the abluminal membrane domains and thus preserving the polarity of the BBB. Potential routes across the BBB for drugs and other solutes are shown in Fig. 2.

Tight junction formation, the polar expression of transport proteins and a full differentiation of the cerebral endothelium, appears to be induced by a close association between the endothelial cells, the adjacent pericytes, and the end-feet of astroglia whose cell bodies lie deeper in the brain parenchyma (Kacem et al., 1998; Dore-Duffy, 2003). The astrocytic end-feet form a network surrounding the abluminal surface of the cerebral endothelial cells, with only the extracellular matrix (basement membrane) separating the cells. It is thought that this close association of the endothelial cells and the astrocytes in particular are responsible for inducing BBB properties and differentiation in the cerebral endothelial cells. There is still much debate about the factors involved which induce this differentiation, but it is likely that they are multiple and some are soluble and some depend on cell-to-cell contact involving molecular handshakes; thus, the cells within the association in turn influence each other. Nerve endings also terminate against the abluminal membrane of the BBB endothelial cells and may influence BBB differentiation and permeability (Rennels et al., 1983). In addition, the endothelial cells, the pericytes, and the astrocytes contribute to the proteins of the extracellular matrix and this structure in turn influences the behavior and differentiation of the cells forming the neurovascular unit (Abbott, 2002). The extracellular matrix immediately surrounding the cerebral endothelial cells and the pericytes is distinct in that it contains laminins 8 and 10, whereas the extracellular matrix of the brain parenchyma contains laminins 1 and 2 (Sixt et al., 2001). Perivascular macrophages and microglia derived from blood macrophages may also form a significant part of the BBB neurovascular unit and contribute differentiating and modulatory signals (Zenker et al., 2003).

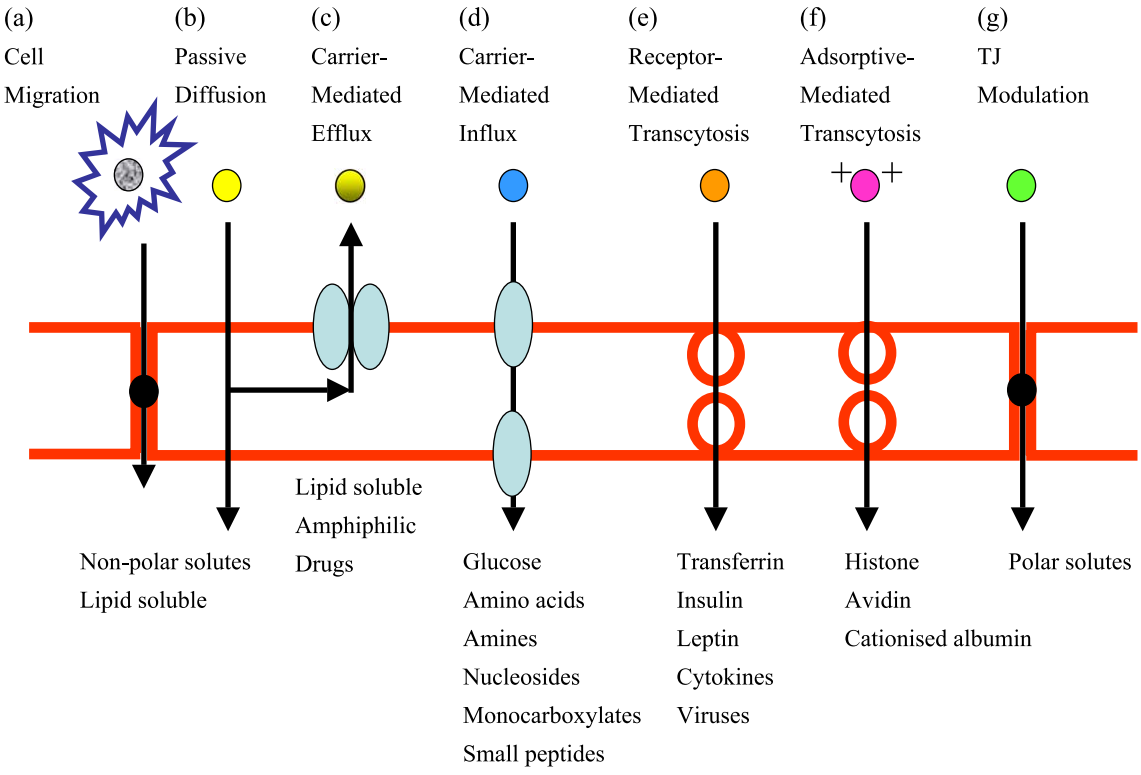


Fig. 2. Potential routes for transport across the BBB. (**a**) Leukocytes may cross the BBB adjacent to, or by modifying, the tight junctions. (**b**) Solutes may passively diffuse through the cell membrane and cross the endothelium. Greater lipid solubility favors this process. (**c**) Active efflux carriers may intercept some of these passively penetrating solutes and pump them out of the endothelial cell. (**d**) Carrier-mediated influx, which may be passive or secondarily active, can transport many essential polar molecules such as glucose, amino acids, and nucleosides into the CNS. (**e**) RMT can transport macromolecules such as peptides and proteins across the cerebral endothelium. (**f**) AMT appears to be induced nonspecifically by negatively charged macromolecules and can also result in transport across the BBB. (**g**) Tight junction modulation may occur, which "relaxes" the junctions and wholly or partially opens the paracellular aqueous diffusional pathway. From Begley and Brightman (2003).

There is also the analogous blood-CSF barrier formed by the epithelia of the choroid plexuses (Wolburg et al., 2001) and around the other circumventricular organs (CVO). The capillaries in the choroid plexus and the CVO do not form tight junctions and the blood vessels in these structures are freely permeable. Indeed, the CVO possess specialized structures in the capillary wall termed fenestrae where the cytoplasm is attenuated and only the plasma membrane(s) form the diffusional barrier (Prestcott & Brightman, 1998; see Fig. 3). In the choroid plexus, the epithelial (ependymal) cells facing the CSF form tight junctions between adjacent cells, thus preventing a free diffusional pathway across this structure. Similarly, the other CVO are surrounded by ependymal cells, which from tight junctions and prevent diffusion of blood-borne solutes further into the brain. Normally, ependymal cells do not form tight junctions elsewhere in the brain. The origin and the mechanisms of the differentiating factors bringing about tight junction formation in the choroid plexus and in ependymal cells near the CVO are quite unknown. The function of the choroid plexus is to secrete fresh CSF and to regulate the movement of solutes from blood to CSF and vice versa. Hence, the plexus epithelial membrane is also polarized in terms of the transporters expressed in the apical and basolateral membranes. The CVO probably form "windows" in the brain so that a restricted volume of brain extracellular fluid is allowed to rapidly equilibrate with plasma (further diffusion within the brain is limited by the tight junctions in the surrounding ependyma). Dendritic processes and receptors on neurons within this limited area can then interact with these blood-borne solutes and their nervous activity can be modulated. These CVO-proximate neurons can then synaptically influence and initiate quite distant events elsewhere in the CNS depending on where their axons terminate. This function of the CVO becomes vital because of the creation of the BBB, and without their monitoring activity, the brain could not rapidly respond to many blood-borne signals. Some of the CVO are termed neurohemal organs and are specialized to allow neurosecretion in the opposite direction from the brain to blood (Fig. 3). Examples of neurohemal organs are the median eminence, the posterior pituitary, and the pineal, which have no BBB and allow neurohormones to easily escape via their fenestrated and open capillaries into the systemic circulation to exert somatic endocrine functions.

The cells forming the avascular arachnoid membrane, which envelopes the whole CNS, also possess tight junctions that effectively seal the paracellular diffusional pathway between these cells.

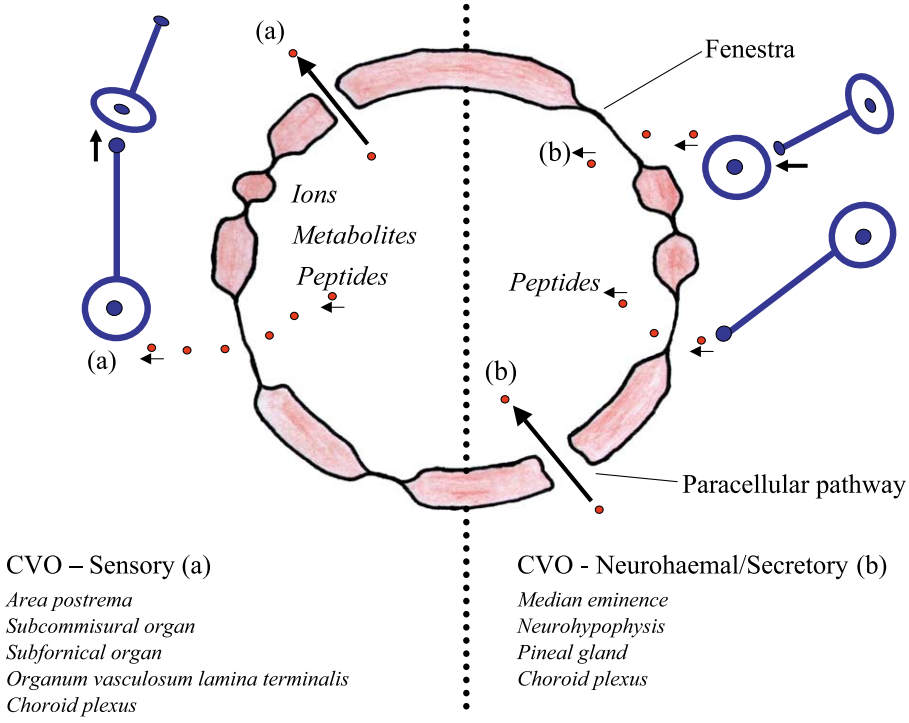


Fig. 3. The organization of a CVO. In the CVO, the capillaries have no tight junctions as well as possess fenestrae where the cytoplasm is extremely attenuated and the opposing cell membranes appear to meet. The absence of tight junctions and the fenestrae ensure that the diffusional resistance of these capillaries is low and the extracellular fluid immediately surrounding the CVO can equilibrate with plasma and vice versa. An ependymal layer surrounding the CVO, in the case of the choroid plexus, the epithelium of the plexus, ensures that only the extracellular fluid immediately around the CVO is in free communication with plasma. The CVO can be divided into those that are primarily sensory in function (**a**) and those that are neurohemal and comprise specialized regions allowing neurosecretion directly into blood (**b**). In the sensory CVO, blood-borne signals can diffuse into the organ and stimulate or inhibit sensory neurons. These neurons may then synapse with other neurons and initiate CNS activity remote from the sensing CVO. In the neurohemal CVO, neurosecretion may be released by nerve endings adjacent to the permeable capillaries, which can then diffuse directly into blood and produce peripheral somatic effects. Note that the choroid plexus may act in both sensory and secretory capacities.

Lipophilic solutes can diffuse through the endothelial cell membrane and enter the CNS passively (Levin, 1980). There is well-established relationship between lipid solubility, either calculated or determined as an oil-water partition coefficient, with brain penetration, which increases with increasing lipid solubility. However, some lipid-soluble molecules do not enter the brain as readily as their lipophilicity solubility might suggest (Fig. 4). These substances and many of their metabolites are removed from the brain and the cerebral endothelium by active efflux transporters, which hydrolyze ATP and can move their substrates against a concentration gradient from blood to brain (Begley, 2004a, 2004b). These active transporters are generally called ATP-binding cassette (ABC) transporters.

The ability of the BBB, the choroid plexus, and the pericytes to transform and detoxify many substances entering the CNS has probably been underestimated in the past, as the transforming enzyme activity of the brain as a whole has usually been considered, which is low, rather than the activity of specific cell types or associations. For several hydrolyzing and conjugating enzymes, the enzyme activity in the choroid plexus per unit weight of tissue is similar to that in the liver (Minn et al., 2000).

Thus, the function of the BBB is essentially 2-fold. It enables the creation of a separate and extremely stable intracerebral extracellular fluid compartment consisting of the CSF and the brain ISF, the composition of which can be maintained distinct from the somatic extracellular fluid.

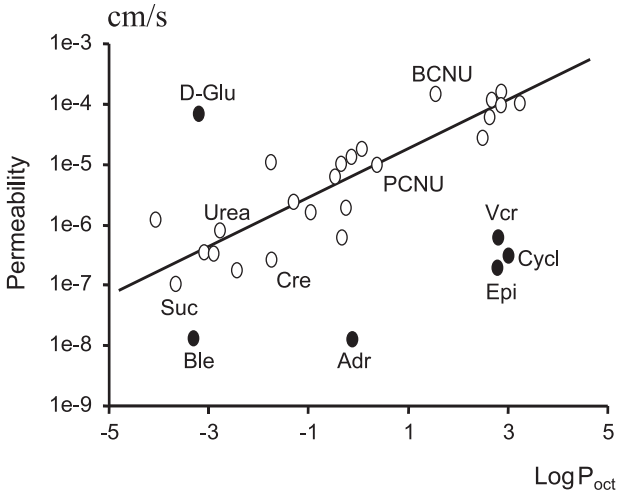


Fig. 4. Graph of BBB permeability (cm/sec) for several solutes plotted against lipid solubility determined in an octanol/water partition system. For many of these solutes (open points), there is a clear correlation between lipid solubility and BBB penetration. There are several outliers shown as filled points. Glucose has a greater BBB penetration than its lipid solubility would suggest as the result of its facilitated transport across the cerebral endothelium. The filled points well below the regression line are all substrates for efflux transporters, principally Pgp. Suc, sucrose; Cre, creatinine; PCNU, 1-(2-chloroethyl)-3-(2,6-dioxo-1-piperidyl)1-nitrosourea; BCNU, 1,3-bis(2-chloroethyl)-*N*-nitrosourea; D-Glu, D-glucose; Ble, bleomycin; Adr, adriamycin (doxorubicin); Epi, epipodophyllotoxin (etoposide); Cycl, cyclosporin A; Vcr, vincristine. Adapted from Levin (1980).

Table 1
A comparison of the concentration of some solutes in CSF and plasma

Solute	CSF	Plasma
Total amino acids (mM)	0.89	2.89
Glucose (mM)	5.38	7.19
Albumin (mg/mL)	0.155 ± 0.039	28.4–53.8
IgG (mg/mL)	0.012 ± 006	9.87 ± 2.2
Total protein (mg/mL)	0.433 ± 790	70.00
Osmolarity (mOsmol)	298.5	305.2
HCO_3^- (mM)	22.0	25.0
pH	7.27	7.46

In total, the amino acid concentration in CSF is ~30% of that in plasma, although for individual amino acids the difference may be much less, with the CSF level being much closer to that of plasma. CSF glucose levels in CSF are consistently lower than plasma. This is partly the result of the rate of glucose penetration through the BBB and partly due to the high rate of glucose utilization by the CNS. Protein levels are always much lower in CSF than in plasma with albumin being 0.004% to 0.002% and immunoglobulin G (IgG) 0.001% of their levels in plasma. Generally, the larger the molecular weight of a plasma protein, the lower its concentration in CSF, suggesting that they "leak" nonspecifically into CSF in a slow but size-dependent manner. The osmolarity of CSF is a little less overall than plasma and the bicarbonate concentration is lower making the pH more acid.

Within these protected compartments, the composition of the extracellular fluid can be precisely regulated in terms of solute concentrations (Table 1). This stability is essential as the CNS relies on accurate synaptic transmission and inhibition, and spatial and temporal summation, to perform its complex integrative functions. Unless the synapse can operate against an extremely stable background, accurate synaptic transmission and nervous integration becomes impossible (Begley & Brightman, 2003). The somatic extracellular fluid contains many potential neurotransmitters and other neuroactive substances whose concentrations in this fluid may vary widely within short periods of time. The CNS could not tolerate, and continue to function, against such a background of significant variation in the concentrations of neuroactive substances that occurs in the general extracellular fluid on a regular basis. Amino acids that are present in blood in high concentration (e.g., glycine, glutamic acid, and aspartic acid) are potent excitatory neurotransmitters; thus, their background concentration in brain extracellular fluid must be maintained stable at very constant levels.

Secondly, the BBB has a neuroprotective function. In a highly complex tissue such as the CNS, where neuronal cell division is either absent or a rare event, any acceleration in cell death and neuronal attrition will cause premature degenerative disease and pathology. Many potentially neurotoxic substances are being continuously ingested in the diet or are generated by metabolism. The BBB is therefore crucial in limiting the access of these potentially damaging xenobiotics and metabolites to the CNS by either blocking their entry or actively removing them from brain via the ABC transporters (Begley & Brightman, 2003; Begley, 2004a, 2004b).

The BBB clearly changes in several brain diseases and a variety of pathological processes may either alter the quality of the barrier or contribute to the development of the disease process (Neuwelt, 2004). Examples of BBB dysfunction in

disease states are defective transport of amyloid-β by the BBB in Alzheimer's disease (Zlokovic, 2004); leptin resistance in obesity, where leptin feedback is impaired as the result of reduced leptin transport across the BBB (Banks & Lebel, 2002); and opening of the barrier in active CNS lesions in multiple sclerosis (Werring et al., 2000) and in brain tumors, where the effects on the cerebral endothelium appear to be varied within the tumor type; it may appear normal and continuous with tight junctions or remain continuous but develop fenestrations or become discontinuous, with or without the development of fenestrations (Schlageter et al., 1999). Different regions within the same tumor may indeed show markedly different changes in microvessel morphology.

The robustness of the BBB may decline with age, although few studies are available (Preston, 2001). Tight junction integrity appears to be maintained with age and a study in aged Fischer 334 rats indicates that P-glycoprotein (Pgp) expression in the BBB is maintained (Warrington et al., 2004). However, well-documented changes in drug pharmacokinetics with age may well alter brain penetration of many drugs and enhance drug-drug interactions.

2. Optimizing the physicochemical properties of central nervous system drugs

The majority of drugs that are used to treat CNS disease have a molecular weight between 150 and 500 Da and a log octanol/water partition coefficient between −0.5 and 6.0 (Bodor & Buchwald, 2003). It is generally assumed that charged molecules cannot readily penetrate the BBB; thus, for a drug that is partially ionized at physiological pH 7.4, it is the uncharged fraction that determines the diffusion gradient across the BBB and forms the driving force for any passive diffusive movement of drug.

Molecular characteristics, which reduce molecule penetrance through the BBB, are a significant polarity, a polar surface area in excess of 80 $Å^2$, a high Lewis bond strength, and a high potential for hydrogen bond formation. In addition, molecules of a given molecular weight, which contain rotatable bonds and those that are highly branched, have a reduced penetration of the BBB (Doan et al., 2002). Relatively small chemical modifications to a molecule may enhance the circulatory half-life and increase the area under the curve in plasma. This increase in half-life may stem from a reduced peripheral distribution volume or resistance to enzymatic hydrolysis in the circulation (Begley, 1996).

There is a clear relationship between the lipid solubility of a drug and its CNS penetration (Levin, 1980), which is thought to represent a direct correlation between BBB penetrance and the ability of a drug to partition into the lipid of the cell membrane (Fig. 4). Thus, designing a drug with optimal lipid solubility for BBB penetration and which retains a significant central pharmacological activity would be the desired solution, but this is often not possible. Simply increasing the lipid solubility of a drug molecule may have undesirable effects such as decreasing solubility and bioavailability, increasing plasma protein binding (often with a high affinity), and increasing uptake by the liver and reticuloendothelial system (RER).

A significant number of molecules have a measured CNS penetration, which is not commensurate with that predicted on the basis of their lipid solubility. For example, several polar molecules such as D-glucose, L-amino acids, and nucleosides penetrate the brain far more readily than their lipid solubility might suggest (Fig. 4). These molecules together with many others, which are essential metabolites for the brain, have a carrier-mediated entry that is either a facilitated diffusive entry or an energy-dependent concentrative mechanism dependent on specific carrier proteins inserted into the luminal and abluminal membranes of the capillary endothelial cells (Begley & Brightman, 2003).

In addition, several highly lipid soluble molecules whose CNS penetration would be expected to be significant do not have the expected high BBB penetration. These drugs were initially thought to be excluded from the CNS by virtue of their physical properties, as they are generally all bulky molecules of high molecular weight. However, it is apparent that not all of the lipid-soluble molecules that are excluded from the CNS share these same characteristics. It is now clear that these drugs are substrates for the ABC group of efflux transporters mentioned earlier, which continually hydrolyze ATP and are able to extrude drugs from the cerebral capillary endothelial cells and the CNS into blood against a concentration gradient. These ABC transporters include Pgp (Pgp/ABCB1), multidrug resistance proteins (MRP/ABCC1–12), and breast cancer resistance protein (BCRP/ABCG2; Begley, 2004a, 2004b).

Several studies in recent years have taken computational in silico approaches to predicting BBB permeability by using several measured or calculated physicochemical variables for a structure to derive a general mathematical equation to predict BBB penetration. The great benefit of this approach would be the early elimination of unsuitable structures and the high-throughput screening of the huge numbers of compounds that can be produced by combinatorial chemistry. This approach to predicting BBB permeability has been reviewed recently (Sipple, 2002). At present, this type of analysis cannot predict compound reactivity with inwardly or outwardly directed transporters in the BBB, which will cause permeability to deviate from the predicted value, or accurately predict pharmacokinetics, which will determine BBB exposure to the drug.

3. Prodrugs and chemical delivery systems

A prodrug approach to delivery to the CNS involves the administration of the drug in a form that is inactive, or weakly active, but is readily able to penetrate the BBB. Ideally, the prodrug should be fairly lipid soluble so that it

penetrates the BBB with ease and is converted into the active form solely within the CNS. Ideally, the active form of the molecule should be more polar than the prodrug so that it effectively becomes locked into the CNS with the consequence that brain levels of the active drug can remain high in the CNS when peripheral levels of the prodrug have declined markedly. An excellent example of this is illustrated by the series of compounds morphine, codeine, and heroin. Their brain uptakes determined by the brain uptake index (BUI) technique (Oldendorf, 1970) are illustrated in Fig. 5. Morphine, although an effective analgesic, does not enter the CNS readily and its brain entry is at the limit of quantification with this technique. Substituting one of the hydroxyl groups in the morphine molecule, thus forming codeine, increases lipid solubility and significantly increases brain uptake. The further substitution of 2 acetyl groups to form acetyl morphine produces a very substantial increase in brain penetration. Thus far, this is an excellent example of increasing brain penetration by chemical lipidization. However, once within the brain, the diacetyl morphine is rapidly metabolized to 6-acetyl morphine and then back to morphine, and it is in this form that it interacts with opioid receptors within the brain. Thus, heroin is acting as a prodrug for morphine within the CNS. It rapidly penetrates into the brain, produces the "rush" that the heroin addict craves, but is effectively acting as morphine. Once converted back into morphine, it is again polar and effectively becomes locked into the CNS, as it cannot easily back diffuse across the BBB; thus, morphine delivered in this way maintains significant CNS levels after the plasma levels of heroin and its metabolites have fallen.

Clearly, several variants of the prodrug approach can be applied. The prodrug may simply have a higher lipid solubility favoring entry or its half-life or stability in plasma may be extended, thus enhancing and maintaining the diffusion gradient of active drug into brain over an extended period. The lipid moiety may consist of a vector attached to the drug by a linker such as an ester or disulfide bond, which is subsequently enzymatically cleaved within the brain; this type of linkage has been termed a chemical delivery system (Bodor & Buchwald, 2003; Bodor & Brewster, 1991). It is estimated that some 5% of listed drugs act as prodrugs (Bodor & Buchwald, 2003), which are subsequently converted into an active metabolite at their site of action. They almost certainly have not been deliberately designed as prodrugs and this particular mode of action, involving a crucial metabolic conversion in the target tissue, has just been serendipitously chanced upon.

4. Intracerebral injection/infusion

One very obvious method for circumventing the BBB is to directly inject a drug, either into brain parenchyma or intraventricularly or intrathecally into CSF. This approach may also be used to introduce a slow-release implant or a colony of stem cells into the brain. A major drawback and a danger with this approach is that any solid implant will

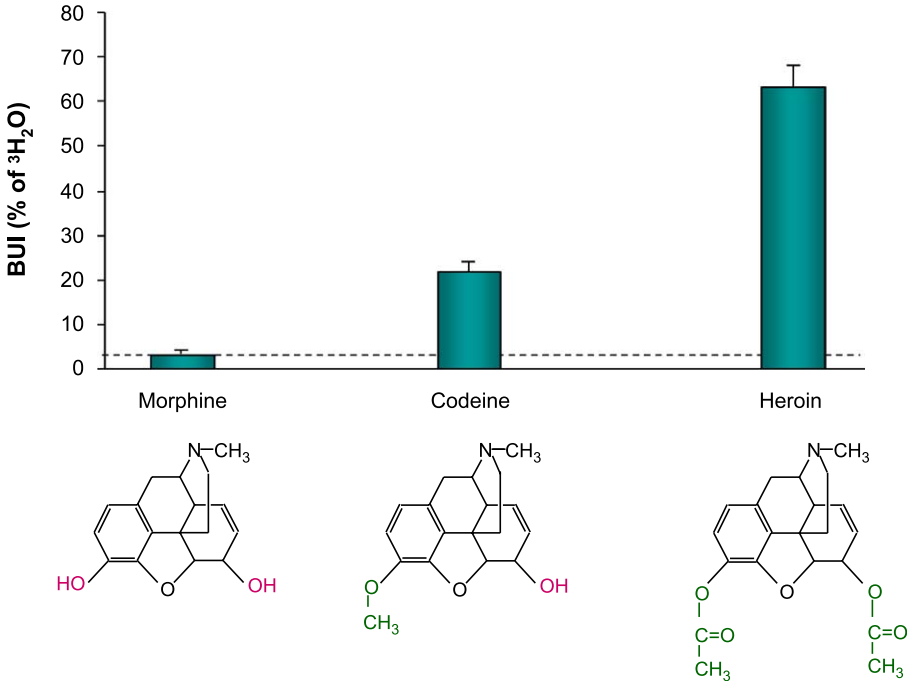


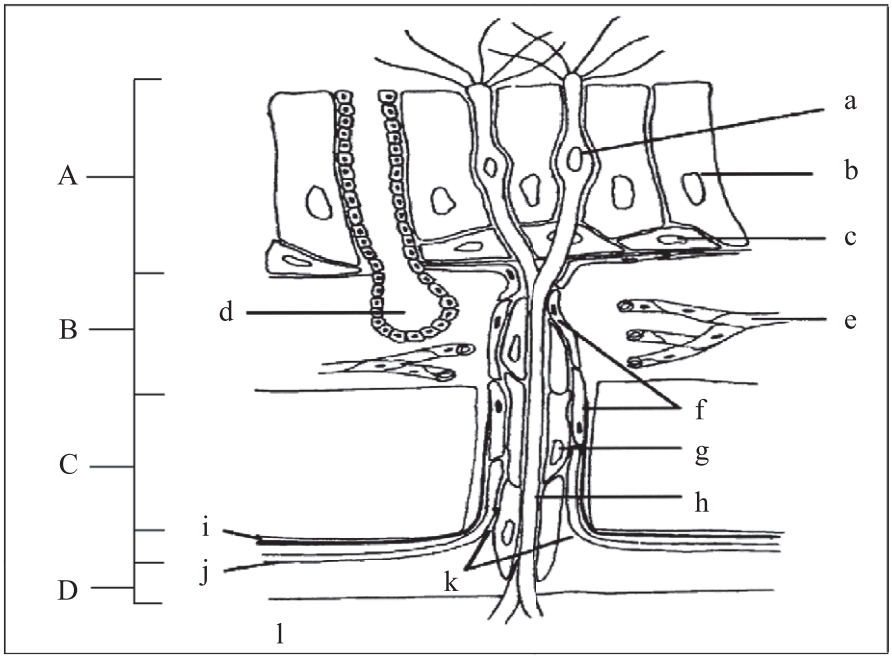
Fig. 5. The BUI of the series: morphine, codeine, and heroin; each of which exhibits a progressive increase in lipid solubility. In the BUI technique, a bolus containing radioactive drug and tritiated water is injected into a carotid artery of the rat and the brain uptake of the drug is expressed as a percentage of the tritiated water uptake (Oldendorf, 1970). Substituting one of the hydroxyl groups of morphine with a methyl group increases the BUI from ~2 to ~20%; replacing both with acetyl groups (diacetyl morphine) increases the BUI to ~65%.

damage brain tissue both around the implant and along the track of a introductory catheter, and a rapid volume injection directly into brain parenchyma will almost certainly damage a volume of brain equal to, or greater than, the volume introduced. This route might be useful for a longer-term slow infusion of drugs, which will gently displace brain extracellular fluid but will always be attended by the risk of introduced infection via an indwelling infusion cannula. Experiments where ^{125}I-labeled nerve growth factor (NGF) preadsorbed onto a small plastic disk, which is then implanted into a rat brain and which will release the NGF slowly over a period of time, have indicated that the growth factor concentrations are negligible only 1 mm or so distant from the disk (Krewson et al., 1995). In addition, when ^{125}I-labeled brain-derived neurotrophic factor (BDNF) was injected intraventricularly, little radioactivity could be found beyond the ependymal cells lining the injected ventricle (Yan et al., 1994). Even if a drug is introduced into brain parenchyma by direct injection, the steady turnover of newly secreted brain extracellular fluid will, by a process of bulk flow, carry the injected drug steadily away from the injection point (Cserr et al., 1981; Cserr & Patlak, 1992; Begley et al., 2000; Begley, 2004a). Similarly, injection into the ventricles will be subject to dilution and a flushing out of the ventricular system by the continuous production of new CSF by the choroid plexuses (Begley et al., 2000; Begley, 2004a). A better knowledge of the direction of these pathways of flow might be applied to deliver drug to specific regions of the brain, especially if the drug can be applied upstream in the flow pathway.

5. The olfactory route

A route into the CNS via the olfactory epithelium and nerves is a viable and interesting possibility for the delivery of some types of drug to the brain (Okuyama, 1997; Illum, 2003).

The olfactory neurons penetrate the cribiform plate and are surrounded by a sleeve of arachnoid membrane, which contains subarachnoid CSF between the nerve and the membrane. This sleeve then terminates in an open-ended manner as the olfactory sensory endings, which penetrate through the olfactory mucosa (Mathison et al., 1998; Fig. 6). The CSF contained in these arachnoid sleeves appears to move outward into the lamina propria and to drain into the local lymphatic system (Bradbury et al., 1981). However, a significant fraction of this fluid appears to recirculate back into the subarachnoid CSF and may carry drug applied to the olfactory mucosa back into the subarachnoid space of the CNS (Begley, 2003; Begley & Brightman, 2003). An alternative hypothesis is that nasally administered drug is taken up by the olfactory nerves themselves and transported by retrograde axonal cytoplasmic flow back into the CNS (Begley, 2003; Illum, 2003). However, a cellular mechanism involving cytoplasmic flow as the major route of transport would probably be much slower than is actually observed, certainly with the drugs that have thus far been investigated, all of which appear in CSF within a few minutes of introduction into the nasal cavity (Sakane et al., 1991a, 1991b, 1995; Illum, 2003). Several drugs have been successfully delivered to the CNS by the nasal route



A: olfactory Mucosa
B: lamina propria
C: cribiform plate
D: sub-arachnoid space

a: olfactory neuron
b: supporting cell
c: basal cell
d: Bowman's gland
e: lymphatic capillaries
f: perineural cells
g: Schwann's cells
h: fila olfactoria
i: dura
j: arachnoid
k: sub-arachnoid sleeve
l: olfactory bulb

Fig. 6. The olfactory route into the CNS. Diagram of the olfactory neurons (a) penetrating the cribiform plate (C). The arachnoid membrane (j) forms a sleeve (k), which encloses the olfactory neurons as they pass through the cribiform plate and the lamina propria (B). This sleeve is open ended at the base of the olfactory mucosa (A). Schwann cells, which have no barrier function, also surround the olfactory neurons. Thus, the subarachnoid space (D) is continuous with the extracellular space of the olfactory mucosa. From Mathison et al. (1998).

including several sulfonamides (Sakane et al., 1991a), cephalexin (Sakane et al., 1991b), progesterone (Anand Kumar et al., 1982), zidovudine (Seki et al., 1994), and several peptides including the hormone insulin and hyaluronidase (Okuyama, 1997; Fehm et al., 2000).

Transport to the brain via the nasal route is enhanced by an increasing lipophilicity of the transported molecule, which suggests that transmembrane movement may be one of the steps in the process of drug transport (Sakane et al., 1991a). Experiments with fluorescently labeled dextrans have also shown that there is an apparent molecular weight cut-off for these tracers of between 20 and 40 kDa (Sakane et al., 1995). However, in spite of these caveats, some relatively large peptides and even viruses and bacteria can enter the brain via the nasal route, which may form an important route for the introduction of CNS infective agents such as meningococcus.

6. Blood-brain barrier modulation

Modulating the efficacy of the tight junctions between the cerebral endothelial cells, so that the paracellular route of access to the brain is either partially or completely opened, is an approach that has been used to permeabilize the BBB to drugs and enhance brain penetration.

Osmotic opening of the barrier is a technique that has been successfully applied over several years in the treatment of human brain tumors (Neuwelt et al., 1991; Rapoport, 2000). The osmotic agent usually employed is hypertonic mannitol. A 25% solution is infused into a carotid artery (in the human at a rate of 4–8 mL/sec) over a period of 30 sec. This treatment opens the barrier rapidly and it remains open for up to 30 min. If a drug is then administered through the same cannula while the barrier is open, it can freely diffuse into the CNS. The hypertonic solution is thought to osmotically pull water out of the endothelial cells, causing cell shrinkage, which may cause disengagement of the extracellular domains of the proteins forming and regulating the tight junctions.

During the treatment of brain tumors, a 10- to 100-fold increase in the delivery of methotrexate (MTX) can be achieved in the region of the tumor when employing an osmotic opening of the BBB. At least some of the paracellular pathways appear to fully open during osmotic opening and larger particles such as viruses may enter the CNS. Opening of the paracellular pathways is, of course, nonselective under these circumstances, and albumin and excitatory neurotransmitters and other potentially damaging substances may gain entry from blood to brain (Begley & Brightman, 2003).

The BBB can be permeabilized by the peptide bradykinin acting via B_2 receptors expressed in the luminal membrane of the endothelium. This action of bradykinin is thought to modulate the tight junctions by elevating intracellular free calcium levels. This free calcium then activates the actin/myosin system within the cell, which shortens, and being linked to the scaffolding (ZO1, ZO2, and ZO3) proteins attached to the junctional proteins, occludin and claudin, may partially withdraw them from the cell membrane and thus modify the properties of the tight junctions. An analogue of bradykinin, RMP7 or Cereport, has been developed as an agent for BBB opening (Emerich et al., 2001). However, opening of the BBB in this way remains relatively nonselective and may admit several damaging plasma solutes such as excitatory amino acids as well as the desired therapeutic drug. As bradykinin receptors are widely distributed in the body, the possibility of undesirable side effects with this approach always remains a possibility.

Alkylglycerols have also been shown to modulate the BBB (Lee et al., 2002; Erdlenbruch et al., 2003). They have to be administered via the carotid artery in a similar manner to mannitol when employed in osmotic opening. The CNS delivery of MTX is significantly increased if coinjected with an alkylglycerol or introduced as a rapidly following bolus (Erdlenbruch et al., 2003). Both monoacetyl and diacetyl glycerols are effective, with 1-*O*-hexyldiglycerol being the most effective. The BBB appears to be rapidly opened and returns to its normal state within 120 min. The general toxicity of the alkylglycerols appears to be low. The mechanism of BBB modulation is at present not certain, and both normal brain and tumor BBB are opened, in contrast to osmotic opening that appears to act preferentially on the BBB of normal brain (Erdlenbruch et al., 2003). The BBB opening is again presumably nonselective. Similar work has also been conducted (Lee et al., 2002) using 1-*O*-hexyldiglycerol and 1-*O*-heptyltriglycerol and it is suggested that BBB disruption with both agents results from the formation of vesicles at the cell membrane by these nonionic detergents and that these vesicles cause pores to form in the plasma membrane of the cerebral endothelial cells, thus allowing polar solutes to freely enter the brain.

Recently, ultrasound and electromagnetic radiation have been employed as modulators of BBB function (Schirmacher et al., 2000; Cho et al., 2002). The mechanism by which the BBB might be modulated by electromagnetic fields is still a matter of debate and some conflicting results appear in the literature as changes in BBB permeability after similar treatments are not always seen (Fritze et al., 1997). At high field intensities, a significant heating of the tissue occurs and this may modify the integrity of tight junctions; however, some effects on BBB integrity are seen at much lower field strengths. A local heating effect can also be produced by ultrasound application. An increased BBB permeability to several solutes may be the result of changes in membrane fluidity or due to alterations in the molecular conformation of transporters resulting in changes in their activity. These thermal and electromagnetic radiation-induced modifications in BBB function and integrity appear to be rapidly induced and are rapidly reversible.

A very attractive feature of BBB modulation with these methods employing ultrasound and microwave electromagnetic fields is that they can be focused with some precision to a particular brain region or to a tumor, thus

selectively modulating the BBB at a preferred site and not globally throughout the brain.

7. Delivery via endogenous transporters

As described above, a large number of solute transporters are present in the BBB (Begley & Brightman, 2003). Many of these transporters are designed to carry polar metabolites into the brain that would otherwise have minimal access to the CNS (see Fig. 2). The expression of these carriers is often polarized to optimize substrate transport into the brain. Thus, with a knowledge of the stereochemical requirements for transport by these carriers, it is possible to design several potential drugs as pseudosubstrates for the transporters and thus enhance their uptake into brain (Tsuji, 2000). Examples of a variety of drugs that can use transporters in the BBB to access the CNS are shown in Table 2. Some of these transporters are very selective in their stereochemical requirements for substrates and they will not transport pseudosubstrates with anything like the affinity and capacity of the endogenous substrates. A good example in this respect is the BBB hexose transporter GLUT1, which is very stringent in its stereochemical requirements for transport. The nucleoside transporter will also not accept a significant number of antiviral analogues and drugs directed at adenosine receptors based on the nucleoside structure (Chishty et al., 2004).

Several peptides are also transported across the BBB by endogenous mechanisms, which are poorly characterized in terms of their transport requirements, and these transport systems may be exploitable (Kastin & Pan, 2003). These peptide transporters, which may be distinct from endocytic mechanisms (see below), may be useful for the therapeutic delivery of peptides and proteins to the brain, especially neurotrophic factors.

The transport system that appears to accept the widest variety of pseudosubstrates is the large neutral amino acid carrier (or L-system) and this carrier has been shown to transport several drugs into the brain (Smith & Stoll, 1999). The transporter recognizes a carboxylic acid group and an amino group covalently linked to the same carbon atom, which is characteristic of an α amino acid or, in the case of baclophen and gabapentin, a conformation that closely resembles this grouping. The transporter is selective for large neutral amino acids in that it also requires the presence of a bulky hydrophobic side group on the molecule, allowing interaction with the cell membrane in such a way that it allows the amino and carboxylic groups of the amino acids to correctly align with the active site of the transporter. In this way, other amino acids such as glycine and alanine are excluded from the transporter (Smith & Stoll, 1999). The antimitotic drug, D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (D,L-NAM), actually has a much higher affinity for the L-system than the natural substrates and is preferentially transported into brain. Some drugs using the L-system such as dihydroxyphenylalanine (L-DOPA) have an affinity for the transporter, which is lower than the endogenous amino acids, and are thus at a competitive disadvantage for transport.

The principle of using endogenous transport mechanisms expressed at the BBB can be also applied to large peptides and proteins that may use either RMT or AMT to carry the peptide/protein across the cerebral endothelium (Bickel et al., 1994). RMT requires binding of a peptide/protein to a receptor on the luminal, plasma facing surface, of the BBB. This receptor-ligand binding then induces an endocytic event in the luminal membrane that probably involves aggregation of receptor-ligand complexes and triggers the internalization of an endocytic vesicle containing the receptors and the attached protein molecules. These internalized vesicles can then enter a pathway, which carries them across the endothelial cell during which the peptide/protein is dissociated from the receptor and exocytosed at

Table 2
Drug entry into the CNS via endogenous transporters

Medium-chain fatty acid carrier
Valproic acid
Docosahexanoic acid (DHA-) taxol
DHA-ddC
Large neutral amino acid carrier
L-DOPA
α-Methyl-DOPA
Melphalan
Baclophen
Gabapentin
Acivicin
D,L-NAM
Phosphonoformate-tyrosine conjugate
Nitrosoarginine derivatives
Monocarboxylic acid carrier
Active metabolites of simvastin and lovastatin (with carboxylic acid groups)
Basic drugs: cation transporter (OCT)
Mepyramine
Diphenhydramine
Diphenylpyraline
Lidocaine
Imipramine
Propranolol
Purine carrier
Oxazolamine COR3224
Nucleoside carrier
Abacivir
Hexose carrier
Dehydroascorbic acid
Glycosylated morphine

DHA-ddc, docosahexanoic acid-2′ ,3′ -dideoxycytidine.

the luminal membrane of the endothelial cell, resulting in transport across the BBB. Not all internalized vesicles are transcytosed and some may enter a pathway that causes them to fuse with a lysosome forming a secondary lysosome, which then constitutes a dead-end, and may result in hydrolysis of the contained peptide/protein (Broadwell et al., 1998). In AMT, there is thought to be a charge interaction between the peptide/protein and the luminal surface membrane of the endothelial cell, which directly induces vesicle formation and internalization. An excess positive charge is especially effective in this respect; thus, cationized albumin and other cationic peptides/proteins that possess a significant positive charge may be transcytosed in this way (Pardridge et al., 1990; see Fig. 2).

An example of the way in which RMT may be used for CNS drug delivery is provided by the use of monoclonal antibodies (mAb; OX26) to the transferrin receptor, which is abundantly expressed in the luminal membrane of the BBB. The OX26 antibody may then be used as a vector for preferential CNS delivery and can be linked to a drug or biologically active peptide/protein, which doe not normally cross the BBB. The binding of the mAb to the transferrin receptor then appears to induce endocytosis and the entire construct is transcytosed across the BBB (Bickel et al., 1994). At the BBB, transferrin receptors are expressed on both the luminal and the abluminal membranes, with a greater abundance on the luminal membrane (Fishman et al., 1987); however, transcytosis of the whole complex of receptor, mAb, and attached peptide appears to occur (Bickel et al., 1994) rather than an exclusive recycling of receptor back to the luminal membrane. By employing a mAb to the transferrin receptor as the vector rather than transferrin protein itself, the strategy avoids the endogenous transferrin in blood competing for transferrin receptors at the BBB. By using an OX26 vector, vasoactive intestinal polypeptide, NGF, glial cell-derived neurotrophic factor, and BDNF have all been successfully delivered to the CNS (Bickel et al., 1994). The capacity of this system for delivery is generally quite low, as the use of a vector in this manner results in only 1 molecule of peptide/protein being delivered per OX26 antibody.

8. Inhibition of efflux mechanisms (ATP-binding cassette transporters)

As mentioned previously, the BBB contains several ABC transporters, which expel a multiplicity of drugs from the CNS (Begley, 2004a, 2004b). Two strategies have emerged for avoiding the activity of these efflux transporters: either by developing specific inhibitors for the efflux transporters, thus giving their substrates a greater access to the CNS, or by attempting to design analogues of drugs with known efficacy but with poor BBB penetration due to ABC transporter activity, which will no longer have a reactivity with the efflux transporters. For both of these strategies to be really effective, it requires a detailed knowledge of the structure-activity relationships (SAR) of the ABC efflux transport mechanisms. This detailed information is proving difficult to obtain. ABC transporters do not interact with their substrates and inhibitors in a

Table 3
Modulators of ABC transporters

First generation Inhibitors	Target
Probenecid	MRP1/2
Sulfinpyrazone	MRP1/2
Benzbromarone	MRP1/2
Verapamil	Pgp
Quinidine	Pgp
Cyclosporin A	Pgp
Second generation Inhibitors	
SDZ-PSC833	Pgp
Third generation Inhibitors	
GF120918	Pgp/BCRP
LY335979	Pgp
V-104	Pgp
Pluronic L-61	Pgp
Fumitremorgin C	BCRP
Experimental agents	
MABs	
Liposomes/nanoparticles	
Oligonucleotides	

Examples of Increased CNS Delivery

Inhibitor	Drug
PSC833	Vinblastine
	Colchicine
	Digoxin
	Paclitaxel
	Morphine-6-glucuronide
GF120918	Colchicine
	Vinblastine
	2', 3'-Didioxyinosine
	Amprenivir
	Itraconazole
LY335979	Paclitaxel
	Nelfinavir

Listed are several inhibitors of ABC transporters and the principal transporters against which they are active. They enhance the CNS uptake of several drugs by inhibiting efflux activity. The inset in the box gives some examples of drugs, where an enhancement of CNS penetration has been demonstrated by the use of 3 of these inhibitors.

Table 4
Physicochemical characteristics if some antihistamines (H1 antagonists)

	log*D*	Molecular weight
First-generation antihistamines (sedating)		
Mepyramine	1.43	285
Imipramine	2.33	280
Hyroxyzine	2.87	375
Diphenhydramine	1.58	256
Second-generation antihistamines (nonsedating)		
Terfenidine	4.46	472
Astemizole	3.48	459
Cetirizine	1.04	389
Temalestine	3.19	442

The first-generation antihistamines are sedating and are not Pgp substrates. The second-generation nonsedating antihistamines are substrates for this efflux pump.

classic enzyme-substrate/lock-and-key manner and therefore standard Menten-Michaelis kinetics cannot be readily applied to their activity (Begley et al., 2000; Martin et al., 2000; Sauna & Ambudkar, 2001; Ambudkar et al., 2003; Begley, 2003; Chang, 2003; Litman et al., 2003; Begley 2004a, 2004b).

Several inhibitors, both competitive and noncompetitive, have been developed to modulate the activity of the major ABC transporters Pgp, MRP, and BCRP. These are shown in Table 3. They have in several instances been successfully used to enhance the uptake of several drugs by the CNS. These are also summarized in Table 3. Although the use of inhibitors of ABC transporters clearly can enhance the BBB uptake of drug substrates, this down-modulation of efflux transport activity may however allow other toxic substrates to more freely enter the brain; thus, long-term use may not be advisable or possible.

It has been shown recently that some of the newer second-generation antihistamine drugs are substrates for Pgp, whereas the earlier antihistamines, which had sedating properties, were not (Chishty et al., 2001; Chen et al., 2003; Polli et al., 2003). Thus, in designing nonsedating properties into these molecules, reactivity with Pgp has also been unintentionally introduced into the molecular structure. Comparing the sedating and nonsedating antihistamines, all of the nonsedating Pgp-interacting antihistamines are larger and more lipophilic and contain overall an increased number of aromatic groups, although clear-cut SAR are not readily apparent (Table 4). A greater understanding of the interaction of ABC transporter substrates with the transporters is required so that informed drug design can proceed to design reactivity with ABC transporters into, or out of, the structure. It is interesting to note that some strategies used to enhance passive permeation of cell membranes, for example, lipidization or an increase in the number of aromatic groups in the molecular structure, may also predispose a molecule as a Pgp substrate and may become self-defeating.

9. Cell-penetrating peptide vectors

Several cell-penetrating peptides, which appear to enter cells with alacrity, have been developed recently (Wadia & Dowdy, 2002; Zhao & Weissleder, 2004). At present, little is known about the mechanism by which these peptides can cross the cell membrane. Some studies suggest that the peptides by virtue of their structure are able to "worm" their way directly through the cell membrane (Derossi et al., 1996; Vivés et al., 1997). They may thus be able to penetrate the cell membrane without causing damage, in a similar manner to a signal peptide entering the endoplasmic reticulum and carrying with it a nascent protein during the normal process of protein synthesis and post-translational modification. One of these cell-penetrating peptides, transactivating-transduction (TAT), acts in the process of replication of the HIV virus

Table 5
Examples of 2 cell-penetrating peptides

Penetratin	RQIKIWFQNRRMKWKK *Antennapedia homeodomain*
SynB1	RGGRLSYSRRRFSTSTGR *Murine protegrin*

Amino acids can be D-isomers to limit enzymic degradation
Amphipathic with separated positivly charged and hydrophobic domains
Repeating amino acid sequences
Engineered to be linear α-helix (no S-S)
Penetrate cell membranes without cytolytic effect
Hydrophobic domains may "worm" into cell membrane
Positively charged regions may interact with negative charges on phospholipid heads
Mechanism similar to signal peptides

Note that the peptides contain repeating sequences of charged and lipophilic amino acids in the structure. This is characteristic of all cell-penetrating peptides. The inset lists some properties of these peptides.

by penetrating the nuclear membrane and acting as an activator of transcription. It has been suggested that the TAT peptide induces the formation of reverse micelles as an energy-independent process (Torchilin et al., 2000), as low temperature and metabolic inhibitors appear to have no influence on the process, and it is also suggested that cell surface receptors play no part in translocation (Wadia & Dowdy, 2002). Other studies suggest that cell-penetrating peptide may induce an endocytic event at the plasma membrane perhaps with a similar mechanism to that of AMT (Green et al., 2003; Richard et al., 2003).

Cell-penetrating peptides consist of an amphipathic α-helix and contain alternating and discrete hydrophobic domains and positively charged domains, which are created by repeating sequences of a charged amino acid such as arginine or lysine followed by a series of hydrophobic residues (Begley, 1996; Rousselle et al., 2000; Bodor & Buchwald, 2003). Examples of 2 cell-penetrating peptides and their properties are shown in Table 5.

These 2 cell-penetrating peptides, penetratin and SynB1, when linked to doxorubicin, have been shown, in a rat in situ brain perfusion model, to increase CNS levels of doxorubicin by 3 to 8 times compared with doxorubicin alone (Rousselle et al., 2000), and TAT has been shown to carry heterologous proteins into several cell types (Fawell et al., 1994) and across the BBB (Schwarze et al., 1999).

10. Liposomes and nanoparticles

Liposomes and nanoparticles are large and complex constructs which can be made from a variety of chemical constituents and may range up to 500 nm in diameter. Relatively large amounts of drug or agent can be incorporated into these structures, providing the possibility for significant delivery to the CNS. The surface of the liposome or nanoparticle can be modified and groups can be attached so that the construct can be targeted to the CNS via specific BBB mechanisms.

Pegylated immunoliposomes have been employed to target and nonpermanently transfect β-galactosidase (LacZ reporter gene) and luciferase into the brain (Shi et al., 2001; Pardridge, 2002). The gene is incorporated into the center of the liposome and the surface of the liposome is then coated with polyethylene glycol (PEG) to prolong the circulation time by reducing uptake of the liposomes by the RER. In addition, a further 2% of the PEG strands have a mAb to the transferrin receptor (8D3 mAb) attached to them (Fig. 7). The mAb then interacts with transferrin receptors and effectively targets the immunoliposome to tissues that have a high expression of transferrin receptors such as the liver and the brain. If a brain-specific promoter is encapsulated with the β-galactosidase gene, for example, the promoter for glial fibrillary acidic protein (GFAP), then the enzyme expression becomes confined to the brain. Using this approach, both β-galactosidase and luciferase have been targeted to the brain and the relevant enzyme is expressed (Shi et al., 2001). The transfection is not permanent and is maximal at 2 days when the GFAP promoter is used. The mechanism by which the immunoliposome carries the gene across the BBB and transfects it into brain cells is not known. An initial step may be the endocytosis of the immunoliposome after binding to the transferrin receptor in an analogous manner to the OX26 vector (Huwyler & Pardridge, 1998).

Similar immunoliposome constructs using OX 26 mAb on the surface have been used to deliver digoxin to the CNS

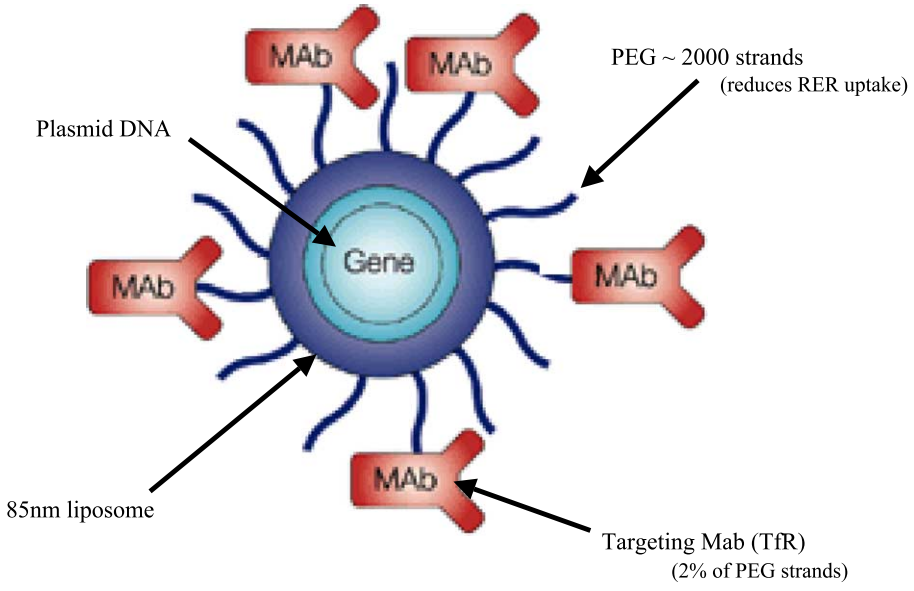


Fig. 7. An immunoliposome. A plasmid DNA containing a gene is packaged into the center of an 85 nm diameter liposome. The surface of the liposome is coated with ~2000 strands of PEG, which reduces uptake by the RER. Between 1% and 2% of these strands are conjugated to the transferrin receptor targeting mAb. From Pardridge (2002).

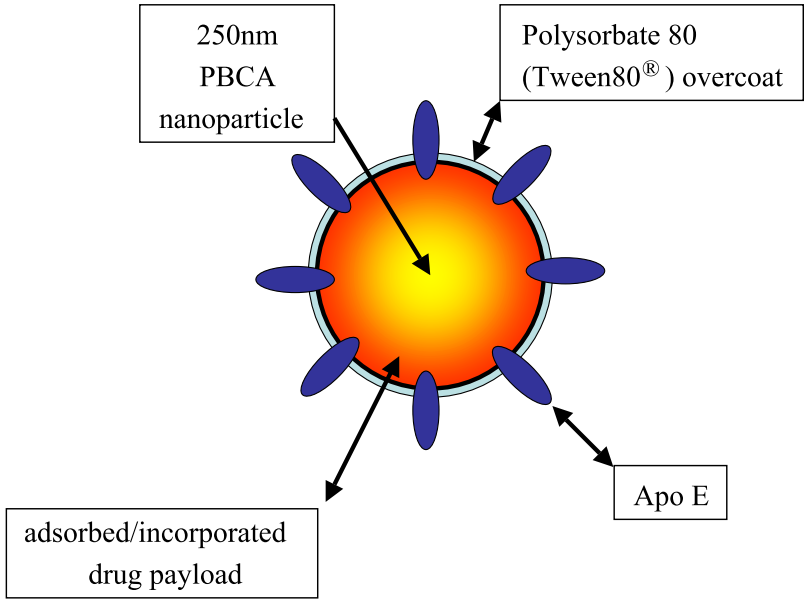


Fig. 8. A PBCA nanoparticle. A drug can be incorporated into the 250 nm diameter nanoparticle during polymerization or absorbed onto the surface of the preformed particle. The particle is then coated with polysorbate 80 (Tween 80), which further binds Apo-E in the bloodstream.

(Huwyler et al., 2002). As digoxin is a Pgp substrate, the inclusion of the drug into immunoliposomes appears to bypass this efflux system and carry the digoxin into the CNS.

Poly(butyl)cyanoacrylate (PBCA) nanoparticles have also been used to deliver drugs to the CNS with a good degree of success (Alyautdin et al., 1995, 1997, 1998; Gulyaev et al., 1999; Alyautdin et al., 2001; Kreuter, 2001; Steiniger et al., 2004). These particles are typically 250 nm in diameter. The nanoparticles are loaded with drug either by incorporating the drug during the initial particle polymerization process or by absorption onto the surface of the preformed particle (Fig. 8). The particles are then coated with Tween 80 (polysorbate 80; Ramge et al., 2000). When they are injected i.v., the surface of the particles becomes further coated with absorbed plasma proteins especially apolipoprotein E (Apo-E). It is thought that this final product is mistaken for low-density lipoprotein (LDL) particles by the cerebral endothelium and is internalized by the LDL uptake system (Kreuter et al., 2002, 2003). Other surfactants are active in producing protein binding by the nanoparticles, but only Tween 80 appears to preferentially absorb Apo-E (Kreuter, 2001).

Table 6
Examples of drugs delivered to the CNS with PBCA nanoparticles

Drug	CNS action
Dalargin (Alyautdin et al., 1995)	Analgesia
Loperamide (Alyautdin et al., 1997)	Analgesia
Tubocurarine (Alyautdin et al., 1998)	Electroencephalographic changes
Doxorubicin (Gulyaev et al., 1999; Steiniger et al., 2004)	Tumor regression

Drugs that have been successfully delivered to the CNS using PBCA nanoparticles are listed in Table 6.

TAT peptide may also be attached to the surface of both liposomes (Torchilin et al., 2000) and nanoparticles (Lewin et al., 2000) and appears to greatly facilitate their internalization by cells, although nanoparticles and liposomes are relatively complex and large structures.

11. Summary and conclusions

The BBB has historically proven to be an enormous impediment to successful drug delivery to the CNS. The process of drug discovery has, for too long centered, on selecting molecules with activity at a particular site or receptor in the brain with scant regard for whether the molecule can be delivered. In the context of CNS disease, this has resulted in many promising molecules failing in their development to clinical trials simply because they cannot cross the BBB in sufficient quantity to be effective.

An improved understanding of passive permeation of the BBB still has an important role to play. Improved understanding of drug receptors and drug sites of action, together with advances in medicinal chemistry, makes it possible to design potential drugs with greatly enhanced activity and selectivity. Further physicochemical modification of these more potent drugs may provide a small but vital increment in their CNS permeability, which results in a very significant increase in the therapeutic index.

Furthermore, designing drugs with reactivity with an influx or efflux transport system in the BBB will facilitate entry into, or help to keep a drug out of, the CNS as desired. The use of endogenous transport systems is very exploit-

able, as long as the natural substrates for transport do not suffer adverse competitive inhibition.

The use of vectors employing a transporter or acting in a nonspecific manner may find an increased application especially when combined with a nanoparticle or liposome containing the drug(s) for delivery. This type of approach using particulate systems potentially allows large payloads of a drug to be delivered, which may have particular application in the delivery of cytotoxic agents, neurotrophic peptides/proteins, enzymes, gene vectors, and other "difficult" large molecules to the brain.

Approaches that seek to modify the properties of the BBB by increasing the permeability of tight junctions or inhibiting the activity of efflux transporters are probably most suited to short-term treatments, where a single or infrequent exposure to a drug is required. Nonselective opening of the BBB or chemical inhibition of efflux systems in the long-term will permit the entry of a wide range of potential neurotoxins and other agents, which will adversely affect CNS homeostasis and produce unwanted side effects.

Our knowledge of the BBB had advanced considerably in recently years and we now appreciate it not only as a static anatomical barrier to free diffusion but also as a highly complex interface that reacts and interact with a range of blood-borne factors and signals produced within the CNS, which modulate its barrier function and activity. The application of modern molecular and cell biological techniques, coupled with traditional, structural in vivo and in vitro techniques, has enabled us to appreciate the barrier as an active bidirectional transport interface that also has significant metabolic and detoxifying enzymatic activities.

It is armed with this improved knowledge that drug development can move into a more informed and rational phase aimed at optimizing drug design for delivery to the CNS.

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