Neurobiology of Disease 37 (2010) 13–25

2010
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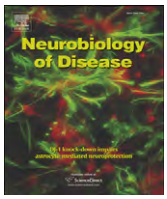
Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

Structure and function of the blood–brain barrier

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ARTICLE INFO

Article history:
Received 9 April 2009
Revised 13 July 2009
Accepted 25 July 2009
Available online 5 August 2009

Keywords:
Blood–brain barrier
Blood–CSF-barrier
Tight junctions
Permeability
Transporters
Receptor-mediated transcytosis
Adsorptive-mediated transcytosis
Drug discovery
Drug delivery
Development
Pathology

ABSTRACT

Neural signalling within the central nervous system (CNS) requires a highly controlled microenvironment. Cells at three key interfaces form barriers between the blood and the CNS: the blood–brain barrier (BBB), blood–CSF barrier and the arachnoid barrier. The BBB at the level of brain microvessel endothelium is the major site of blood–CNS exchange. The structure and function of the BBB is summarised, the physical barrier formed by the endothelial tight junctions, and the transport barrier resulting from membrane transporters and vesicular mechanisms. The roles of associated cells are outlined, especially the endfeet of astrocytic glial cells, and pericytes and microglia. The embryonic development of the BBB, and changes in pathology are described. The BBB is subject to short and long-term regulation, which may be disturbed in pathology. Any programme for drug discovery or delivery, to target or avoid the CNS, needs to consider the special features of the BBB.

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2009.07.030

Introduction

Signalling in the central nervous system

Neurons within the central nervous system (CNS) communicate using a combination of chemical and electrical signals, and precise regulation of the local ionic microenvironment around synapses and axons is critical for reliable neural signalling. It has been argued that this was one of the chief evolutionary pressures leading to the development of mechanisms for maintaining the homeostasis of the neural microenvironment (Abbott, 1992). Barrier layers at the key interfaces between blood and neural tissue play a major role in this regulation (Abbott et al., 2006).

CNS barriers

All organisms with a well developed CNS have a blood–brain barrier (BBB) (Abbott, 2005). In the brain and spinal cord of mammals including humans, the BBB is created by the endothelial cells that form the walls of the capillaries. The combined surface area of these microvessels constitutes by far the largest interface for blood–brain exchange. This surface area, depending on the anatomical region, is between 150 and 200 $cm^2\ g^{-1}$ tissue giving a total area for exchange in the brain of between 12 and 18 m^2 for the average human adult (Nag and Begley, 2005).

A second interface is formed by the epithelial cells of the choroid plexus facing the cerebrospinal fluid, which constitute the blood–cerebrospinal fluid barrier (BCSFB). The CSF is secreted across the choroid plexus epithelial cells into the brain ventricular system (Brown et al., 2004), while the remainder of the brain extracellular fluid, the interstitial fluid (ISF), is derived at least in part by secretion across the capillary endothelium of the BBB (Cserr et al., 1981; Cserr and Patlak, 1992; Abbott, 2004; Dolman et al., 2005). ISF and CSF are free to communicate at several locations; different experimental studies have estimated the contribution of ISF to CSF as 10–60%

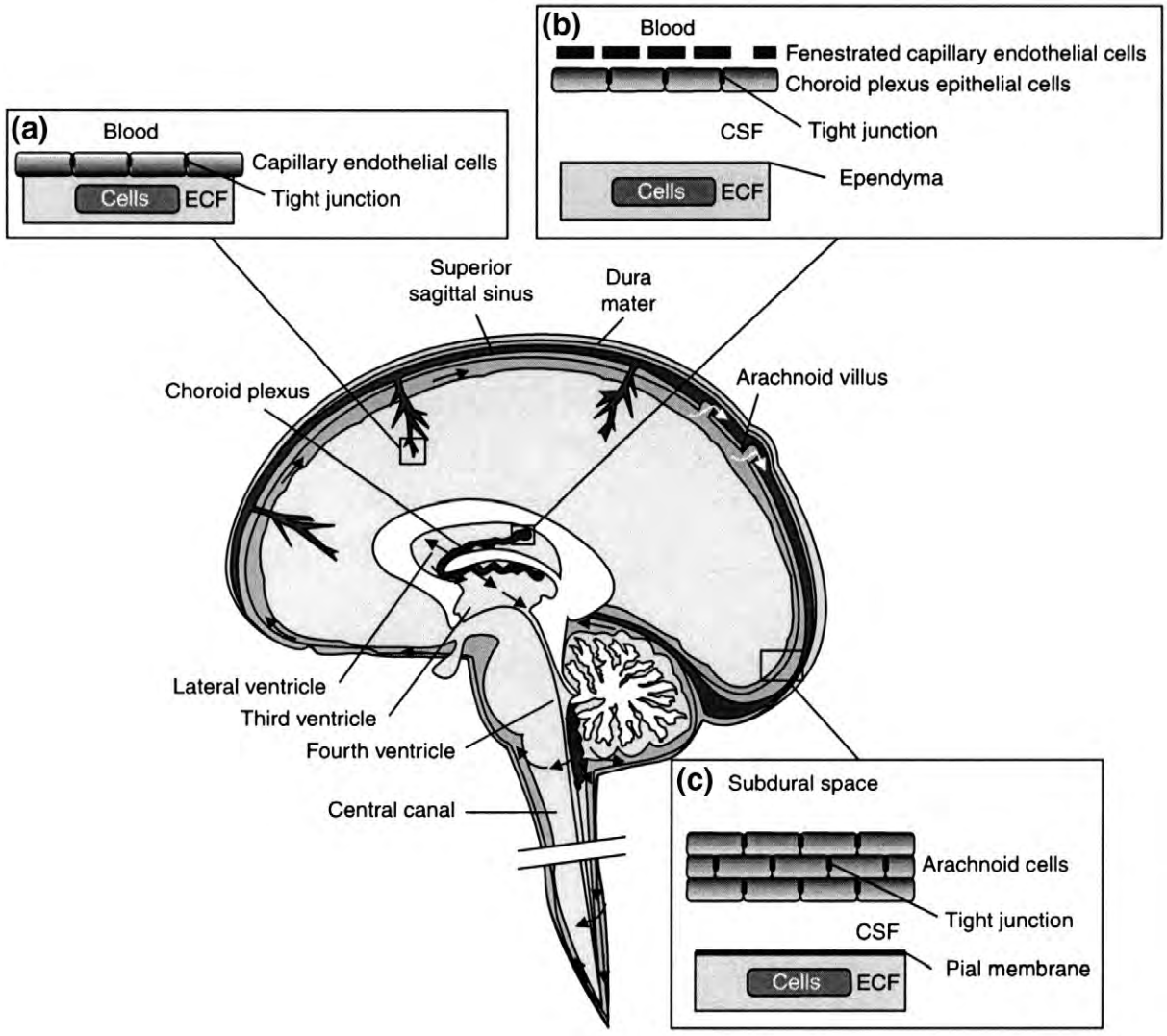

Fig. 1. Barriers of the brain. There are three principal barrier sites between blood and brain. (a) The BBB proper, which is created at the level of the cerebral capillary endothelial cells by tight junction formation. It is by far the largest surface area for exchange and in the adult human is between 12 and 18 m^2 in surface area. No brain cell is further than about 25 μm from a capillary, so once the BBB is crossed, diffusion distances to neurons and glial cell bodies for solutes and drugs are short. Targeting a drug across the BBB is therefore the favoured route for global delivery of drugs to all brain cells. (b) The blood–CSF barrier (BCSFB) lies at the choroid plexuses in the lateral, third and fourth ventricles of the brain where tight junctions are formed between the epithelial cells at the CSF-facing surface (apical surface) of the epithelium. Some drugs and solutes enter the brain principally across the choroid plexuses into CSF, while others enter via both the BBB and BCSFB. (c) The arachnoid barrier. The brain is enveloped by the arachnoid membrane lying under the dura. The arachnoid is avascular but lies close to the superior sagittal sinus and is separated from it by the dura. The arachnoid is a multi-layered epithelium with tight junctions between cells of the inner layer that form an effective seal. Arachnoid villi project into the sagittal sinus through the dura and a significant amount of CSF drains into the sinus through these valve-like villi which only allow CSF movement out of the brain to blood. Transport across the arachnoid membrane is not an important route for the entry of solutes into brain (adapted from Kandel et al., 2000, with permission).

(Milhorat et al., 1971; Davson and Segal, 1995). The secretion of CSF and ISF is driven by the ionic and osmotic gradient created by the Na^+, K^+-ATPase, expressed in the abluminal membrane of the BBB endothelium and the apical membrane of the choroid plexus epithelium, resulting in water movement and volume flow (Abbott, 2004).

The third interface is provided by the avascular arachnoid epithelium, underlying the dura, and completely enclosing the CNS; this completes the seal between the extracellular fluids of the central nervous system and that of the rest of the body (Abbott et al., 2006). Although the arachnoid also forms a barrier layer, its avascular nature and relatively small surface area mean that it does not represent a significant surface for exchange between the blood and the CNS (Fig. 1) (Kandel et al., 2000).

At all three interfaces, the barrier function results from a combination of physical barrier (tight junctions between cells reducing flux via the intercellular cleft or paracellular pathway), transport barrier (specific transport mechanisms mediating solute flux), and metabolic barrier (enzymes metabolizing molecules in transit). The barrier function is not fixed, but can be modulated and regulated, both in physiology and in pathology (Abbott et al., 2006).

Functions of CNS barriers

Ion regulation

The BBB not only provides a stable environment for neural function, but also by a combination of specific ion channels and transporters keeps the ionic composition optimal for synaptic signalling function. Thus the concentration of potassium in mammalian plasma is approximately 4.5 mM, but in CSF and brain ISF this is maintained at ~2.5–2.9 mM, in spite of changes that can occur in plasma $[K^+]$ following exercise or a meal, imposed experimentally, or resulting from pathology (Bradbury et al., 1963; Hansen, 1985). Ca^{2+}, Mg^{2+} and pH are also actively regulated at the BBB and BCSFB (Somjen, 2004; Jeong et al., 2006; Nischwitz et al., 2008).

Neurotransmitters

Blood plasma contains high levels of the neuroexcitatory amino acid glutamate which fluctuate significantly after the ingestion of food. If glutamate is released into the brain ISF in an uncontrolled manner, as for example from hypoxic neurons during ischemic stroke, considerable and permanent neurotoxic/neuroexcitatory damage can occur to neural tissue. Since the central and peripheral nervous systems use many of the same neurotransmitters, the BBB also helps to keep the central and peripheral transmitter pools separate, minimising 'cross-talk' (Abbott et al., 2006; Bernacki et al., 2008).

Macromolecules

The BBB prevents many macromolecules from entering the brain. The protein content of CSF is much lower than that of plasma, and the individual protein composition markedly different (Table 1). Plasma proteins such as albumin, pro-thrombin and plasminogen are damaging to nervous tissue, causing cellular activation which can lead to apoptosis (Nadal et al., 1995; Gingrich and Traynelis, 2000; Gingrich et al., 2000). Factor Xa is present in the brain, which converts prothrombin to thrombin, and the thrombin receptor PAR_1 is widely expressed in the CNS. Similarly tissue plasminogen activator is present in central nervous tissues and converts plasminogen to plasmin. Thrombin and plasmin if present in brain ISF can initiate cascades resulting in seizures, glial activation, glial cell division and scarring, and cell death (Gingrich and Traynelis, 2000). Thus leakage of these large molecular weight serum proteins into brain across a damaged BBB can have serious pathological consequences. One of the few proteins to have a higher concentration in CSF than in plasma is cystatin-C (Table 1), which is synthesised locally within the CNS (Reiber, 2001). Cystatin-C is a serine protease inhibitor and a high concentration in CSF may be a protective measure against micro-leaks in the BBB which continually and spontaneously occur and would otherwise allow plasma components to seep into the brain.

Table 1
Typical plasma and cerebrospinal fluid concentrations for some selected solutes. From Begley (2007).

Solute	Units	Plasma	CSF	Ratio
Na^+	mM	140	141	~1
K^+	mM	4.6	2.9	0.63
Ca^{++}	mM	5.0	2.5	0.5
Mg^{++}	mM	1.7	2.4	1.4
Cl^-	mM	101	124	1.23
HCO_3^-	mM	23	21	0.91
Osmolarity	mOsmol	305.2	298.5	~1
pH		7.4	7.3	
Glucose	mM	5.0	3.0	0.6
Total amino acid	μM	2890	890	0.31
Leucine	μM	109	10.1–14.9	0.10–0.14
Arginine	μM	80	14.2–21.6	0.18–0.27
Glycine	μM	249	4.7–8.5	0.012–0.034
Alanine	μM	330	23.2–32.7	0.07–0.1
Serine	μM	149	23.5–37.8	0.16–0.25
Glutamic acid	μM	83	1.79–14.7	0.02–0.18
Taurine	μM	78	5.3–6.8	0.07–0.09
Total protein	mg/ml	70	0.433	0.006
Albumin	mg/ml	42	0.192	0.005
Immunoglobulin G (IgG)	mg/ml	9.87	0.012	0.001
Transferrin	mg/ml	2.6	0.014	0.005
Plasminogen	mg/ml	0.7	0.000025	0.00004
Fibrinogen	mg/ml	325	0.00275	0.000008
α2-macroglobulin	mg/ml	3	0.0046	0.0015
Cystatin-C	mg/ml	0.001	0.004	4.0

Neurotoxins

The BBB functions as a protective barrier which shields the CNS from neurotoxic substances circulating in the blood. These neurotoxins may be endogenous metabolites or proteins, or xenobiotics ingested in the diet or otherwise acquired from the environment. A number of ABC energy-dependent efflux transporters (ATP-binding cassette transporters) actively pump many of these agents out of the brain (see below). The adult CNS does not have a significant regenerative capacity if damaged and fully differentiated neurons are not able to divide and replace themselves under normal circumstances. There is a continuous steady rate of neuronal cell death from birth throughout life in the healthy human brain, with relatively low levels of neurogenesis (Lim et al., 2007). Any acceleration in the natural rate of cell death resulting from an increased access of neurotoxins into the brain would become prematurely debilitating.

Brain nutrition

The BBB has low passive permeability to many essential water-soluble nutrients and metabolites required by nervous tissue. Specific transport systems therefore are expressed in the BBB to ensure an adequate supply of these substances. The differentiation of the endothelium into a barrier layer begins during embryonic angiogenesis (see below) and in the adult is largely maintained by a close inductive association with several cell types, especially the endfeet of astrocytic glial cells. This induction promotes the upregulation of tight junction proteins and the development of polarity in the endothelial cells arising from the differential expression of specific transporter proteins in the luminal and abluminal membranes (Abbott et al., 2006; Wolburg et al., 2009). Pericytes, microglia and nerve terminals are also closely associated with the endothelium, and play supporting roles in barrier induction, maintenance and function (Abbott et al., 2006; Shimizu et al., 2008; Nakagawa et al., 2009). The cell associations at the BBB are shown in Fig. 2.

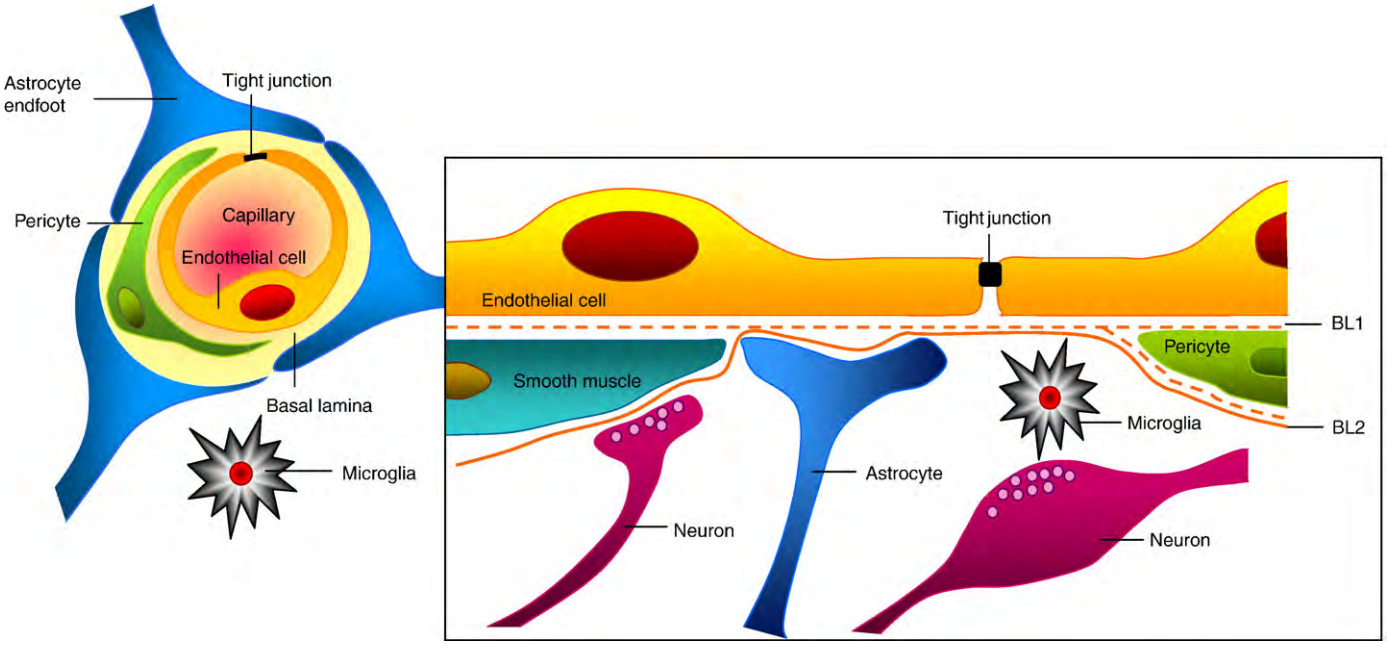

Fig. 2. The cell associations at the BBB. The cerebral endothelial cells form tight junctions at their margins which seal the aqueous paracellular diffusional pathway between the cells. Pericytes are distributed discontinuously along the length of the cerebral capillaries and partially surround the endothelium. Both the cerebral endothelial cells and the pericytes are enclosed by, and contribute to, the local basement membrane which forms a distinct perivascular extracellular matrix (basal lamina 1, BL1), different in composition from the extracellular matrix of the glial endfeet bounding the brain parenchyma (BL2). Foot processes from astrocytes form a complex network surrounding the capillaries and this close cell association is important in induction and maintenance of the barrier properties. Axonal projections from neurons onto arteriolar smooth muscle contain vasoactive neurotransmitters and peptides and regulate local cerebral blood. BBB permeability may be regulated by release of vasoactive peptides and other agents from cells associated with the endothelium. Microglia are the resident immunocompetent cells of the brain. The movement of solutes across the BBB is either passive, driven by a concentration gradient from plasma to brain, with more lipid-soluble substances entering most easily, or may be facilitated by passive or active transporters in the endothelial cell membranes. Efflux transporters in the endothelium limit the CNS penetration of a wide variety of solutes (based on Abbott et al., 2006).

In summary the CNS barriers together provide the stable fluid microenvironment that is critical for complex neural function, and protect the CNS from chemical insult and damage.

BBB tight junctions

The BBB to macromolecules and most polar solutes is created by tight junctions (TJs) between the cerebral endothelial cells, the choroid plexus epithelial cells and the cells of the arachnoid epithelium. Extremely tight 'tight junctions' (*zonulae occludentes*) are a key feature of the BBB and significantly reduce permeation of polar solutes through paracellular diffusional pathways between the endothelial cells from the blood plasma to the brain extracellular fluid (Begley and Brightman, 2003; Wolburg et al., 2009).

The junctional complexes between endothelial cells include adherens junctions (AJs) and TJs. In AJs, cadherin proteins span the intercellular cleft and are linked into the cell cytoplasm by the scaffolding proteins alpha, beta and gamma catenin. The adherens junctions hold the cells together giving the tissue structural support. They are essential for formation of tight junctions, and disruption of AJs leads to barrier disruption (Wolburg and Lippoldt, 2002). The tight junctions consist of a further complex of proteins spanning the intercellular cleft (occludin and claudins), and junctional adhesion molecules (JAMs) (Wolburg and Lippoldt, 2002; Wolburg et al., 2009) (Fig. 3). Occludin and claudins are linked to a number of cytoplasmic scaffolding and regulatory proteins ZO-1, ZO-2, ZO-3 and cingulin. There are some 20 known isoforms of claudin (claudins 1–20) (Mitic et al., 2000). In experimental allergic encephalomyelitis (EAE) and glioblastoma multiforme (GBM) there is a selective loss from the tight junctions of claudin-3, but not claudin-5 or occludin, and this disappearance is associated with a loss of BBB integrity together with some functional barrier loss (Wolburg et al., 2003). In the case of EAE changes in BBB permeability are associated with inflammatory events at the BBB, with disease severity during the acute phase of EAE being directly correlated with extent of BBB permeability (Fabis et al., 2007). Also mice genetically altered to lack claudin-5 have a severely compromised and leaky blood–brain barrier and die shortly after birth (Nitta et al., 2003), although their death is probably not solely related to the BBB defects. Therefore it appears that disappearance of either claudin-3 or claudin-5 from the tight junctional complexes can result in a compromised BBB. The tight junctions of the BBB are sensitive to locally produced CNS and circulating factors, which can on a minute-to-minute basis modulate the properties and the function of the paracellular pathway (Wolburg et al., 2003). The barrier function of the TJs is not solely related to the expression and presence of claudins and occludin spanning the intercellular cleft, but is also influenced by the way these proteins are organised and interact (Hamm et al., 2004). The expression of occludin and JAMs also influences tight junction formation and function (Engelhardt, 2007).

The tight junctions are responsible for the severe restriction of the paracellular diffusional pathway between the endothelial cells to ions and other polar solutes, and effectively block penetration of macromolecules by this route. The impediment to ion movement results in the high *in vivo* electrical resistance of the blood–brain barrier, of ~1800 Ω cm^2 (Butt et al., 1990). This high electrical resistance or low conductance of the potential paracellular pathway emphasises the extreme effectiveness of the tight junctions in occluding this pathway by effectively reducing the movement of ions. The hydrated radius of a sodium ion (Na^+) is 3.6 Å (Volkov et al., 1997). The *in vivo* blood–brain barrier for an extracellular marker such as sucrose (MW 342 Da, molecular radius of 4.7 Å) is extremely effective, with a measured *in vivo* permeability of 2.5–3.0×10^{-8} cm s^{-1} (Ohno et al., 1978). Ionic lanthanum (hydrated radius 4.6 Å, Bouldin and Krigman, 1975), when introduced into the cerebral capillary lumen, can be shown by electron microscopy to penetrate the intercellular cleft as far as the tight junctional complexes and then its movement is arrested (Brightman and Reese, 1969; Bouldin and Krigman, 1975).

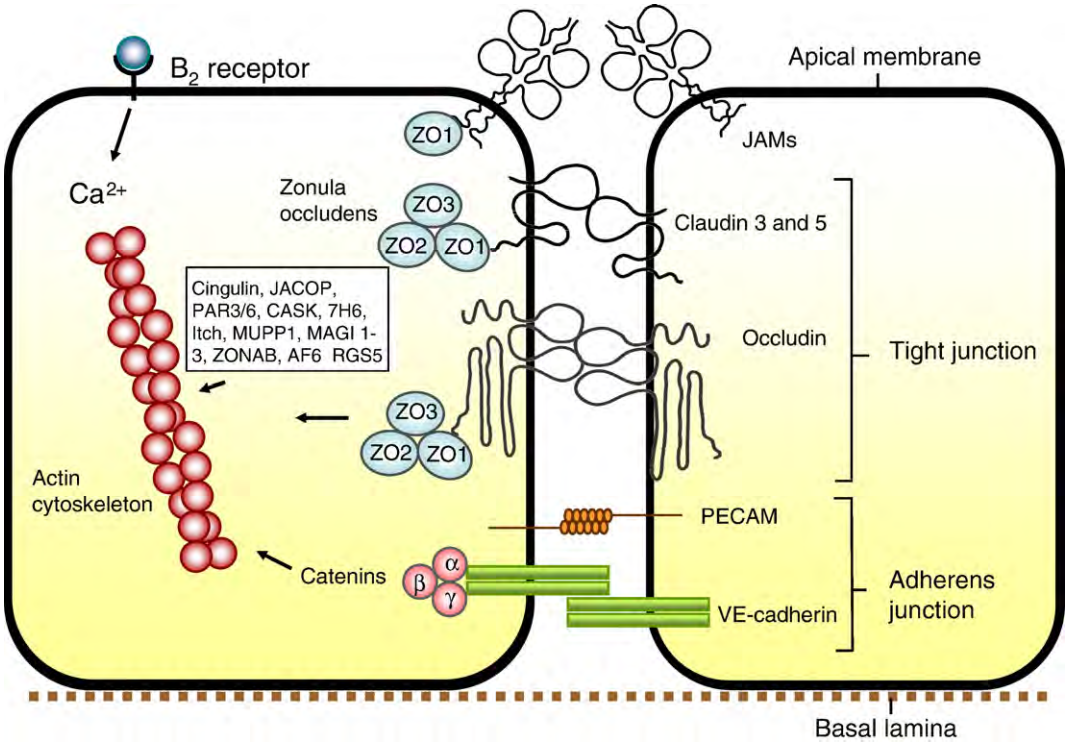

Fig. 3. Structure of BBB tight junctions. The tight junctional complex comprises occludin, claudins 3 and 5, and possibly other claudins. Cadherins of the adherens junctions provide structural integrity and attachment between the cells, and are necessary for formation of tight junctions. The barrier to diffusion and the high electrical resistance of the BBB appear to be largely due to the properties of claudins 3 and 5 (see text). The claudins associate and bind to each other across the intercellular cleft. A different ratio of the claudin mix may subtly alter tight junctional properties and their tightness. Occludin has similar associations across the cleft but does not form the restrictive pore to small ions. The claudins and occludin are linked to the scaffolding proteins ZO-1, ZO-2 and ZO-3, linked in turn via cingulin dimers to the actin/myosin cytoskeletal system within the cell. Activation of the actin cytoskeleton may be initiated by a rise in intracellular calcium, for example resulting from ligand binding to the B_2 bradykinin receptor, and may change the configuration of claudins and occludin thus modifying the tight junctional properties. The role of the junction-associated molecules (JAMs, members of the immunoglobulin superfamily) is unclear, but they appear to act as cell-adhesion molecules for leukocytes (updated from Begley, 2007 and other sources, see text).

The effectiveness of the tight junctions appears to be regulated via the intracellular scaffold proteins ZO-1, ZO-2 and Z0-3 which link the junctional molecules claudin and occludin via cingulin to intracellular actin and the cytoskeleton (Wolburg and Lippoldt, 2002; Bauer et al., 2004; Wolburg et al., 2009). Alterations in both intracellular and extracellular calcium concentration can modulate tight junction assembly (Balda et al., 1991; Abbott, 1998; Abbott et al., 2006), and alter the electrical resistance across the cell layer and the effectiveness of the tight junctions as a barrier. Many of the cell types associated with brain microvessels, including microglia and astrocytes, and nerve terminals adjacent to the endothelial extracellular matrix/basal lamina release vasoactive agents and cytokines which can modify tight junction assembly and barrier permeability (Rennels et al., 1983; Abbott et al., 2006).

Induction and maintenance of many blood–brain barrier properties, including formation of tight junctions and the polarised expression of transporters in the luminal and abluminal endothelial membranes, depends on a close association with astrocytes (Rubin et al., 1991; Abbott, 2002; Wolburg et al., 2009). Tight junction formation between cerebral endothelial cells in culture can be induced by the use of astrocyte-conditioned medium, evidence for action of soluble inducing factors (Neuhaus et al., 1991; Abbott, 2002; Lee et al., 2003). More recently, induction by pericytes, neurons and cells of monocyte lineage has also been described (Abbott et al., 2006; Nakagawa et al., 2009). As several inducing factors for different features of the BBB phenotype have been identified, it is clear that barrier induction involves multiple agents and cell types. Two-way exchange of signalling molecules and induction of some features via the extracellular matrix or close cell:cell interaction add further complexity to the induction process (Abbott, 2002; Begley, 2004a,b; Abbott et al., 2006).

Transport across the BBB

Several potential routes for permeation across the BBB are shown in Fig. 4.

Passive partitioning into brain

A wide range of lipid-soluble molecules can diffuse though the BBB and enter the brain passively (Liu et al, 2004). There is a general correlation between the rate at which a solute enters the CNS and its lipid solubility, usually determined as the logD octanol/buffer partition coefficient at pH 7.4 (Clark, 2003). In contrast with logP which refers to the partitioning of one species, most commonly neutral, logD includes neutral and ionized species present in solution (Krämer, 1999; Waterhouse, 2003). Factors which restrict the entry of compounds into the CNS are a high polar surface area (PSA) (greater than 80 $Å^2$), and a tendency to form more than 6 hydrogen bonds, a factor which greatly increases the free energy requirement of moving from an aqueous phase into the lipid of the cell membrane (Clark, 2003; Gleeson, 2008). The presence of rotatable bonds in the molecule and a molecular weight in excess of 450 Da also appear to restrict BBB permeability. A high affinity of binding to plasma proteins with a low off-rate can also significantly reduce CNS penetration. However, these molecular and physicochemical factors are not always an absolute indication for CNS penetration and activity and there are many examples of effective CNS active drugs in clinical use which do not comply with these general rules for BBB penetration (Bodor and Buchwald, 2003). Bases, which carry a positive charge, have an advantage over acids when penetration of the BBB is considered and it is probably the cationic nature of these molecules and an interaction with the

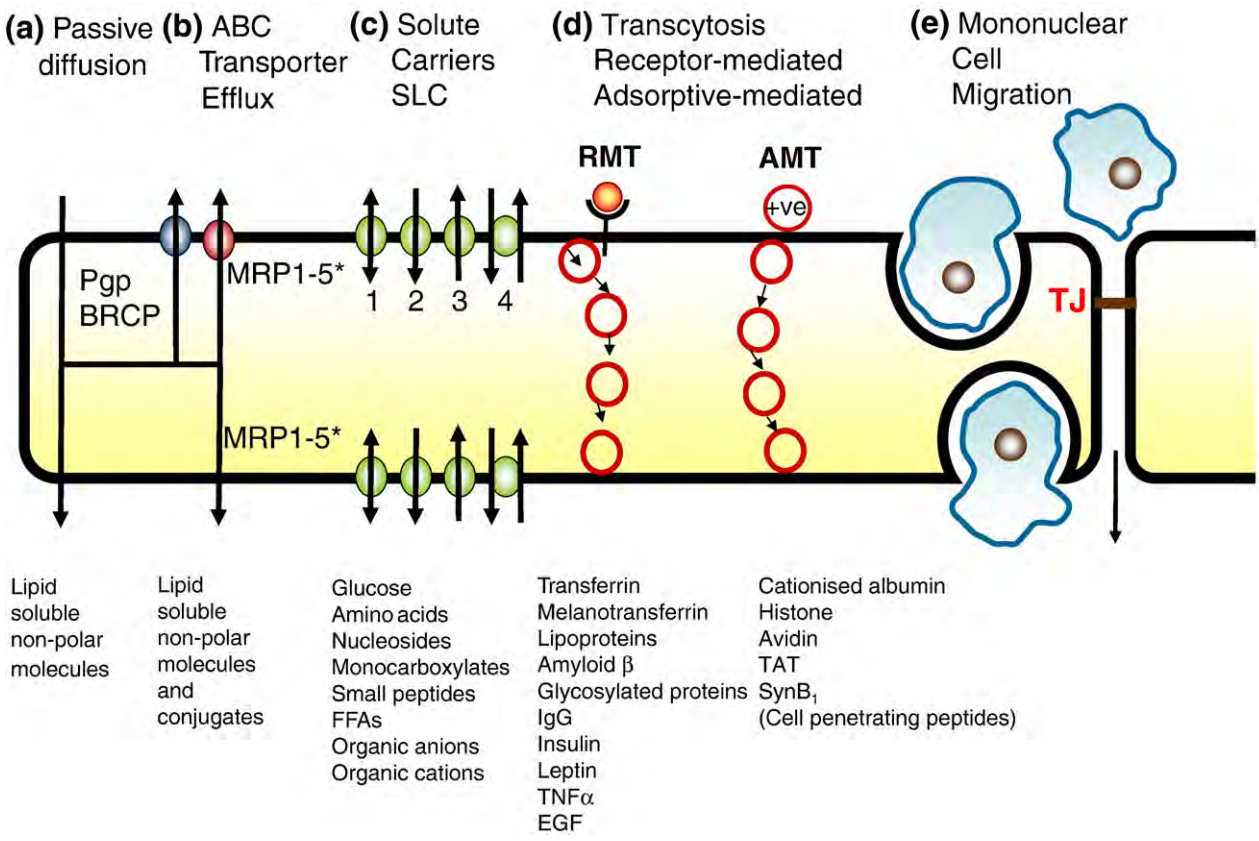

Fig. 4. Routes of transport across the BBB. (a) Solutes may passively diffuse through the cell membrane and cross the endothelium. A higher lipid solubility and several other physico-chemical factors favour this process (see text). (b) Active efflux carriers (ABC transporters) may intercept some of these passively penetrating solutes and pump them out of the endothelial cell either as they diffuse through the cell membrane or from the cytoplasm. Pgp and BCRP are strategically placed in the luminal membrane of the BBB endothelium. MRPs 1–5* are inserted into either luminal or abluminal membranes; there appear to be some species differences in both the polarity and the isoforms of MRPs expressed at the BBB (Begley, 2004a,b). (c) Carrier-mediated influx via solute carriers (SLCs) may be passive or primarily or secondarily active and can transport many essential polar molecules such as glucose, amino acids and nucleosides into the CNS. The solute carriers may be bi-directional, the direction of net transport being determined by the substrate concentration gradient (1), unidirectional either into or out of the cell (2/3), or involve an exchange of one substrate for another or be driven by an ion gradient (4). In this last case the direction of transport is also reversible depending on electrochemical gradients. (d) RMT requires receptor binding of ligand and can transport a variety of macromolecules such as peptides and proteins across the cerebral endothelium (transcytosis). AMT appears to be induced in a non-specific manner by positively charged macromolecules and can also transport across the endothelium. Both RMT and AMT appear to be vesicular-based systems which carry their macromolecule content across the endothelial cells. (e) Leukocytes cross the BBB either by a process of diapedesis through the endothelial cells (penetrating close to the tight junctional regions), or via modified tight junctions. The junction-associated molecules (JAMs) and the cell surface protein CD99 may interact with the leukocytes to initiate diapedesis. Tight junction modulation can result from signals from cells associated with the brain endothelium or be induced pharmacologically; molecular re-arrangement of the proteins of the tight junctions results in complete or partial opening of the paracellular aqueous diffusional pathway (modified from Begley, 2007).

negatively charged glycocalyx (heparan sulphate proteo-glycans) and phospholipid head groups of the outer leaflet of the cell membrane that facilitate their entry.

The movement of the blood gases oxygen and carbon dioxide across the BBB is diffusive and the dissolved gases move down their concentration gradients. Oxygen supply to the brain and carbon dioxide removal are thus blood-flow dependent, so as long as cerebral blood flow remains within physiological limits, gas transport is adequate. The negatively charged bicarbonate ion has a very low passive permeability across the BBB.

Solute carriers (SLCs) in the BBB

The barrier to paracellular diffusion potentially isolates the brain from many essential polar nutrients such as glucose and amino acids necessary for metabolism and therefore the BBB endothelium must contain a number of specific solute carriers (transporters) to supply the CNS with these substances. The formation of tight junctions essentially confers on the BBB the properties of a continuous cell membrane, both in terms of the diffusional characteristics imposed by the lipid bilayer, and the directionality and properties of the specific transport proteins present in the cell membrane. Examples of BBB solute carriers (SLC transporters) are listed in Table 2.

Most polar molecules cannot diffuse through cell membranes and thus all cells express a large number of SLCs in the cell membrane (Zhang et al., 2002). The brain endothelial cells forming the BBB express transport proteins for a wide variety of solutes and nutrients, mediating flux into and out of the brain. Some of these transport proteins are polarised in their expression and are inserted into either the luminal or abluminal membrane only, others are inserted into both membranes of the endothelial cells (Betz et al., 1980; Begley, 1996; Mertsch and Maas, 2002; Abbott, 2002; Begley and Brightman, 2003; Nag and Begley, 2005; Ohtsuki and Terasaki, 2007; Roberts et al., 2008; Bernacki et al., 2008). The orientation of these transporters may therefore result in preferential transport of substrates into or across the endothelial cell and the direction of the transport may be from blood to brain or brain to blood. A further function of the tight junctions in the lateral cell membranes is to act as a 'fence' in the membrane and segregate transport proteins and lipid rafts, to either the luminal or abluminal membrane domain, and to prevent their free movement from one side of the endothelium to the other, thus preserving the polarity of the barrier.

ATP-binding cassette transporters (ABC transporters) in the BBB

When comparing brain penetrance with lipid solubility (lipophilicity) a large number of solutes and drugs have a much lower CNS

Table 2
Some significant solute carriers expressed in the BBB.

Transporter (SLC)	Abbreviation and/or transporter subtype	EquivalentHuman Gene Name	BBB location	Orientation[a]	Example of endogenous substrates/mechanism
Glucose	GLUT1	SLC2A1	Luminal Abluminal	Blood to brain	Glucose (Facilitative, bi-directional)
Sodium-dependent glucose transporter	SGLT1	SLC5A1	Abluminal	Brain to endothelium	Glucose
Sodium myoinositol cotransporter	SMIT HMIT/GLUT13	SLC5A3 SLC2A13	Luminal	Blood to endothelium	Myoinositol (Sodium-dependent)
Cationic L-amino acid transporter	CAT1 (y^+) CAT3	SLC7A1 SLC7A3	Luminal	Blood to endothelium	Basic L-amino acids Lysine, arginine (Sodium-independent)
Large neutral amino acid transporter	LAT1 (system L)	SLC7A5	Luminal Abluminal	Blood to brain	Asparagine, glutamate, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine (Facilitative, bi-directional)
Amino acid	y^+LAT2	SLC7A6	Luminal Abluminal	Blood to brain	Arginine, lysine, ornithine (Facilitative, bi-directional)
Amino acid	y^+LAT2 ? (Na^+-dependent LNAA, system y^+L)	SLC7A6?	Abluminal	Brain to endothelium	Alanine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine (Sodium-dependent)
Amino acid	SNAT2 (System A)	SLC38A2	Abluminal	Brain to endothelium	Small neutral amino acids Alanine, asparagine, proline, serine, glycine (Sodium-dependent)
Amino acid	SNAT 3 SNAT 5 (System N)	SLC38A3 SLC38A5	Abluminal	Brain to endothelium	Asparagine, glutamine, histidine, serine (Sodium-dependent)
Amino acid	System n	?	Luminal	Blood to endothelium	Asparagine, glutamine, histidine (Facilitative)
Amino acid	ASCT1 ASCT2 (System ASC)	SLC1A4 SLC1A5	Abluminal	Brain to endothelium	Alanine, cysteine, glycine, isoleucine, leucine, methionine, serine, threonine, valine (Sodium-dependent)
Amino acid	EAAT1 EAAT2 EAAT3	SLC1A3 SLC1A2 SLC1A1	Abluminal	Brain to endothelium	Anionic amino acids Glutamate, aspartate (Sodium-dependent)
Amino acid	x^-_G	SLC1A?	Luminal	Blood to endothelium	Glutamate (Facilitative)
Amino acid	GLYT	SLC6A9	Luminal?	Blood to endothelium	Glycine Na and Cl dependent
Amino acid	TAUT, β	SLC6A6	Luminal Abluminal	Brain to endothelium	Taurine, β-alanine (Sodium-dependent)
Nucleosides, nucleotides and nucleobases	ENT1 ENT2	SLC29A1 SLC29A2	Luminal	Blood to endothelium	Nucleosides, nucleotides, nucleobases (Facilitative, equilibrative)
Nucleosides, nucleotides and nucleobases	CNT1 CNT2 CNT3	SLC28A1 SLC28A2 SLC28A3	Abluminal	Endothelium to brain	Nucleosides, nucleotides, nucleobases (Sodium-dependent exchange)
Monocarboxylic acids	MCT1	SLC16A1	Luminal Abluminal	Blood to brain	Ketone bodies
Monocarboxylic acids	MCT2 ?	SLC16A7	Abluminal	Brain to endothelium	Lactate (Proton exchanger)
Thyroid hormone transporter	MCT8 (XPCT)	SLC16A2	Luminal Abluminal	Blood to brain	T3 thyroid hormone (Facilitative)
Organic anion transporters	OAT2 OAT3	SLC22A7 SLC22A8	Luminal	Blood to brain	Dicarboxylate exchange with α-ketoglutarate, bicarbonate, Cl^-
Organic anion transporting polypeptide	OATPB/OATP2B1 OATP1A4 OATP1C1	SLCO2B1 SLCO1A4 SLCO1C1	Luminal Abluminal	Blood to endothelium Endothelium to brain	Organic anion/bicarbonate exchangers
Organic cation transporters	OCT2 OCT3	SLC22A2 SLC22A3	Luminal	Blood to endothelium	Organic cation/proton exchange
Novel organic cation transporter	OCTN2	SLC22A5	Luminal Abluminal	Blood to endothelium Endothelium to brain	Organic cation/proton exchange
Amine transporter	PMAT	SLC29A4	Abluminal	Brain to endothelium	Organic cation/proton exchange, MPP^+, serotonin, dopamine
Choline transporter	CTL1	SLC44A1	Luminal Abluminal	Blood to endothelium Endothelium to brain	Choline Facilitative

Note that species differences have been reported for some transporters, and that different cell culture models may express the BBB transporters to different extents. For several additional transporters, gene expression has been shown but functional characterisation is lacking (Dahlin et al., 2009).
? = specific gene name not assigned/orientation not confirmed.
Updated from Begley (2007); see also Kamiie et al., 2008; Lyck et al., 2009; Dahlin et al., 2009.
[a] In order to transport a substrate across the blood–brain barrier from blood to brain the transporter must be expressed in both cell membranes and be bi-directional. Alternatively one transporter may carry substrate into the BBB endothelial cells and another out of the cells. If a transporter is inserted into one membrane only it will transport out of the endothelium or accumulate substrate within the endothelium. Exchangers are driven by the concentration gradient of substrate and exchange ion/molecule and can reverse if the concentration gradient is reversed.

entry rate than might be expected from their logD. These substances and many of their metabolites are actively effluxed from the brain and the capillary endothelium forming the BBB by members of the ABC transporter (ATP-binding cassette) family (Begley, 2004b) (Table 3 and Fig. 4). The strategy of increasing the lipid solubility of a drug to make it more brain penetrant may sometimes be counter-productive as it may also increase the likelihood of the molecule becoming a substrate for ABC efflux transporters (Begley, 2004a,b; Eilers et al., 2008; Förster et al., 2008; Giri et al., 2008). ABC transporters in the human are a superfamily of proteins containing 48 members which on the basis of structural homology are grouped into 7 sub-families (Dean et al., 2001). In the BBB the ABC transporters of greatest significance for efflux transport are P-glycoprotein (Pgp, Multidrug Resistance Protein, ABCB1), the Multidrug Resistance-associated Proteins (MRPs, ABCC1, 2,4, 5 and possibly 3 and 6), and Breast Cancer Resistance Protein (BRCP, ABCG2) (Begley, 2004b; Dauchy

Table 3
Human ABC transporter superfamily and relevance for BBB and BCSFB.

Sub-family	BBB significant members	Alias	Substrates	Expression
ABCA1–12	ABCA2		MDR surfactants	Brain and lung
ABCB1–11	ABCB1	P-glycoprotein Pgp, MDR1 Rodent mdr1a/mdr1b	MDR (amphiphilic/lipid-soluble)	BBB (apical); choroid plexus sub-apical (vesicular)
ABCC1–12	ABCC1	MRP1	MDR/OA/conjugates	BBB luminal and abluminal; choroid plexus
	ABCC2	MRP2/MOAT	MDR/OA/conjugates	
	ABCC3	MRP3	MDR/OA/conjugates	
	ABCC4	MRP4	Nucleosides	
	ABCC5	MRP5	Nucleosides	
ABCD1–4				Peroxisomes
ABCE1				
ABCF1–3				Ubiquitous
ABCG1 + 2; 4 + 5; 8	ABCG2	BCRP/MXR		BBB luminal

OA — Organic acids.
MDR — multiple drug resistance drugs.

et al., 2008; Kamiie et al., 2008) (Table 3). The ABC transporters ABCA1 and ABCG1 transport cholesterol and are also expressed in brain and the BBB. ABCA2 is also expressed in the CNS and has been reported to be associated with drug resistance (Dean et al., 2001), although its functional significance has not been explored.

The major role of the ABC transporters in the BBB is to function as active efflux pumps consuming ATP and transporting a diverse range of lipid-soluble compounds out of the brain capillary endothelium and the CNS. In this role they are removing from the brain potentially neurotoxic endogenous or xenobiotic molecules and are carrying out a vital neuroprotective and detoxifying function (Dallas et al., 2006). In addition many drugs are substrates for these ABC efflux transporters and their brain penetration is significantly reduced (Begley, 2004b) by this transport activity. Pgp and BCRP are expressed in the luminal membrane of the BBB and clearly their function is to transport substrate from endothelium to blood; recent studies suggest some cooperativity of action (Polli et al., 2009). Some of the MRP isoforms appear to be expressed in either the luminal or the abluminal membrane, or sometimes both (Roberts et al., 2008). As they favour water-soluble conjugates as substrates, a bi-directional export from the endothelium may be acceptable, as conjugation by drug transforming enzymes will render them less cytotoxic. There may also be some species variation in the expression of the MRP isoforms (Begley, 2004b). While the brain endothelium is clearly the major barrier interface, the transport activity of both pericytes (Shimizu et al., 2008) and perivascular astrocyte endfeet (Wolburg et al., 2009) may contribute to barrier function under physiological conditions, and may act as a 'second line of defence' if the primary barrier is breached or dysfunctional (see below).

BBB transport of macromolecules

Transcytosis of macromolecules across the BBB via endocytotic mechanisms provides the main route by which large molecular weight solutes such as proteins and peptides can enter the CNS intact. Although the majority of large blood-borne molecules are physically prevented from entering the brain by the presence of the blood–brain barrier and tight junctions, specific and some non-specific transcytotic mechanisms exist to transport a variety of large molecules and complexes across the BBB. A summary of a number of known transcytotic mechanisms is presented in Table 4 (Zlokovic et al., 1990; Pardridge et al., 1990; Pan and Kastin, 1999; Pan et al., 2000; Banks et al., 2002; Demeule et al., 2002; Stern et al., 2002; Talukder et al., 2003; Drin et al., 2003; Herz and Marschang, 2003; Visser et al., 2004; Deane et al., 2004; Banks, 2004; Gaillard et al., 2005) (see also Fig. 4).

These vesicular mechanisms involve either receptor-mediated transcytosis (RMT) or adsorptive-mediated transcytosis (AMT). In RMT the binding of macromolecular ligands to specific receptors on

Table 4
Examples of transcytosis/transport of large molecules and complexes across the BBB.

Transport system	Abbreviation (receptor)	Example ligands	Type	BBB direction	Reference
Transferrin	TfR	Fe-transferrin	RMT	Blood to brain	Visser et al., 2004
Melanotransferrin	MTfR	Melanotransferrin (p97)	RMT	Blood to brain	Demeule et al., 2002
Lactoferrin	LfR	Lactoferrin	RMT	Blood to CSF	Talukder et al., 2003
Apolipoprotein E receptor 2	ApoER2	Lipoproteins and molecules bound to ApoE	RMT	Blood to brain	Herz and Marschang, 2003
LDL-receptor-related protein 1 and 2	LRP1 LRP2	Lipoproteins, Amyloid-β, lactoferrin, α 2-macroglobulin, melanotransferrin (p97), ApoE	RMT	Bi-directional	Herz and Marschang, 2003; Gaillard et al., 2005
Receptor for advanced glycosylation end-products	RAGE	Glycosylated proteins, Amyloid-β, S-100, amphotericin	RMT	Blood to brain	Stern et al., 2002; Deane et al., 2004
Immunoglobulin G	Fcγ-R	IgG	RMT	Blood to brain	Zlokovic et al., 1990
Insulin	–	Insulin	RMT	Blood to brain	Banks, 2004
Leptin	–	Leptin	RMT	Blood to brain	Banks et al., 2002
Tumour necrosis factor	–	TNFα	RMT	Blood to brain	Pan and Kastin, 2002
Epidermal growth factor		EGF	RMT	Blood to brain	Pan and Kastin, 1999
Heparin-binding epidermal growth factor-like growth factor (diphtheria toxin receptor)	HB-EGF (DTR)	Diphtheria toxin and CRM197 (protein)	RMT	Blood to brain	Gaillard et al., 2005
Leukaemia inhibitory factor	LIFRa (gp190)	LIF	RMT	Blood to brain/ spinal cord	Pan et al., 2000
Cationised proteins	+	Cationised albumin	AMT	Blood to brain	Pardridge et al., 1990
Cell penetrating peptides	+	SynB5/pAnt-(43–58)	AMT	Blood to brain	Drin et al., 2003

Many of the receptors involved in RMT are poorly defined and are multifunctional and multiligand in nature. Thus some ligands may be transported by more than one system and some receptors may with time turn out to be one-and-the same.
(− receptor uncharacterised; + not receptor-mediated, non-specific).

the cell surface triggers an endocytotic event. The receptors and their bound ligand cluster together, and a caveolus is formed which pinches off into a vesicle, then both ligand and receptors are internalised into the endothelial cell and routed across the cytoplasm to be exocytosed at the opposite pole of the cell. Dissociation of the ligand and receptor presumably occurs during cellular transit or during the exocytotic event. AMT requires an excess positive charge on the molecule, which renders it cationic, then interaction with cell surface binding sites induces endocytosis and subsequent transcytosis (Sauer et al., 2005). In both cases, to achieve transcytosis of an intact protein or peptide, the lysosomal compartment within the cell needs to be avoided by routing the primary sorting endosome and its contents away from this degradative compartment. Routing away from the lysosome appears not to occur in many peripheral endothelia, and may be a specialised feature of the BBB where the intact transcytosis of a significant number of macromolecules becomes a necessity (Nag and Begley, 2005). By contrast, in many cells and tissues, the contents of the primary endosome are routed to the acidic lysosome where enzymatic degradation takes place (Broadwell et al., 1988; Mellman, 1996; Mukherjee et al., 1997).

Most electron microscopic studies of BBB endothelial cells suggest the presence of relatively few observable endocytotic vesicles in the cytoplasm of these cells compared to other endothelia. For example, the BBB contains only 16–20% of the endocytotic profiles seen in muscle capillary endothelia (Claudio et al., 1989), although they may increase to comparable levels with BBB inflammation (Kastin and Pan, 2003). However, when a comparison is made of the ability of capillary endothelia in a variety of tissues to trancytose protein, there is a poor correlation between the protein permeability of a microvessel and the number of observable endocytotic profiles (Claudio et al., 1989; Stewart, 2000). Brain capillary endothelia are very thin cells, the luminal and abluminal membranes being only separated by ~500 nm (5000 Å) or less. Caveolae are 50–80 nm in diameter and thus the events of transcytosis may be difficult to capture within the cell using conventional electron microscopical techniques.

Smaller peptides may be transported across the BBB by either non-specific fluid-phase endocytosis or RMT mechanisms. It may also be possible for them to use a peptide-specific transporter protein, directly inserted into the cell membrane in a similar manner to the solute transporters, which transfers them through the membrane (Kastin and Pan, 2003; Banks, 2004; Dogrukol-Ak et al., 2009). In some cases the receptor which transduces the signal at the cell membrane may also act as the transporter for the peptide and be co-opted to initiate RMT or another transport system. In other cases the membrane transporter for a peptide may be quite distinct in structure from the receptor which transduces signals at the cell membrane from a signalling peptide (Kastin and Pan, 2003).

Cell movement across the BBB

Cells from the bone-marrow derived monocyte lineage enter the brain during embryonic development and become resident immunologically-competent microglia (Glezer et al., 2007). Mononuclear leukocytes, monocytes and macrophages are able to be recruited to the CNS in pathological conditions, and play roles complementary to those of the resident microglia (Davoust et al., 2008); in some cases they may transform into a microglial phenotype (Hess et al., 2004; Bechmann et al., 2005; Schwartz et al., 2006). Circulating neutrophils and mononuclear cells are attracted to sites of BBB inflammation, penetrate the barrier and form cuffs in the perivascular space around small vessels especially venules; the perivascular space acts as a specific niche for coordinated immune response (Bechmann et al., 2001; Konsman et al., 2007). However, as a result of the BBB and strictly regulated immune cell–BBB interaction, the central nervous system acts as an immune privileged site, and neutrophil infiltration into the brain is low compared to other tissues. Brain penetration occurs only after events such as trauma or ischemia when activated neutrophils damage the BBB (Scholz et al., 2007).

Perivascular macrophages and microglia can become activated in inflammation and other pathological states. In the normal BBB, mononuclear cells appear able to penetrate by a process of diapedesis directly through the cytoplasm of the endothelial cells and not via a paracellular route involving re-arrangement and opening of the tight junctional complexes as had been previously suggested (Engelhardt and Wolburg, 2004; Wolburg et al., 2005). This mechanism enables the mononuclear cells to cross the BBB without TJ disruption. During diapedesis the leukocyte enters the endothelial cell with the luminal membrane closing over it before it creates an opening in the abluminal membrane, so a fluid-filled channel through the cell is never created (Wolburg et al., 2005; Carman and Springer, 2008). In inflammatory pathological states which involve the BBB, the tight junctions between endothelial cells may be opened as a result of actions of cytokines and other agents, and mononuclear cells may then enter by both transcellular and paracellular routes (Anthony et al., 1997; Bolton et al., 1998; Konsman et al., 2007).

Development of the blood–brain barrier

The BBB develops during fetal life and is well formed by birth, especially to proteins and macromolecules (Olsson et al., 1968; Tauc et al., 1984; Saunders, 1992; Moos and Møllgård, 1993; Keep et al., 1995; Preston et al., 1995; Saunders et al., 2000; Ballabh et al., 2004). In the mouse the BBB begins to form between E11 and E17, by which time identifiable tight junctions are present. The presence of TJs will tend to restrict trans-endothelial movement of polar solutes and macromolecules. In mammals born in a relatively immature state, such as the rat and mouse, many of the characteristic BBB transport mechanisms such as ion regulation may continue to mature and only become fully expressed and functional in the peri- or post-natal period (Jones et al., 1992). Recent evidence indicates that at the molecular level, Wnt/beta-catenin signalling controls development of the blood–brain barrier (Liebner et al., 2008).

In the mouse an RT-PCR signal for the ABC transporter mdr2 is present in brain tissue by E13 and all three mdr isoforms, mdr1a, mdr1b and mdr2, show strong signals by E18 (Scheingold et al., 2001). In the rodent the mdr1a and mdr1b isoforms are the principal efflux and drug transporting isoforms. In the human there is only a single MDR P-glycoprotein gene product MDR1 that effluxes and transports drugs (Begley, 2004b); MDR1 in the human and mdr1a in the rodent are both expressed in the luminal membrane of the BBB endothelial cells and mdr1b is expressed by glia and elsewhere in the CNS of rodents (Begley, 2004b). Expression of P-glycoprotein (mdr1a) in the luminal endothelial cell membranes of the rat BBB can be detected by immunoblotting at P7 and reaches a plateau of expression by P28 (Matsuoka et al., 1999).

The high electrical resistance, characteristic of the blood–brain barrier, is exhibited in the BBB of rats by E21 (Butt et al., 1990; Butt, 1995) indicating that functional tight junctions are formed prenatally. This low conductivity demonstrates that they already form an effective barrier to the movement of ions; however, the barrier is significantly tightened on the day of birth (Butt et al., 1990). It has been demonstrated that the BBB permeability to ^{86}Rb is significant at E21 with an influx rate constant of 42.5 ± 4.3 μl g^{-1} min^{-1} but within 2 days post-natally this has declined to 12.2 ± 0.6 μl g^{-1} min^{-1}, and is then followed by a further slow decline to 7.0 ± 0.3 μl g^{-1} min^{-1} by 50 days post-natally (Keep et al., 1995). Tight junction formation appears to be a very early feature of BBB development, and a barrier to the free movement of proteins and macromolecules is formed at the primary stages of brain development (Saunders et al., 2000). Tight junctions are formed as blood vessels invade the brain at E10 in the mouse and E11 in the rat. As gliogenesis in the rat does not begin until E17 and is still occurring post-natally, tight junction formation

appears to be initiated by signals from neurons and progenitor cells rather than from differentiated glia in the first instance (Saunders et al., 2000).

Preston et al. (1995) showed that BBB permeability to the non-metabolisable, but slowly BBB penetrant, tracer mannitol (182 Da) is between 0.19 and 0.22 μl g^{-1} min^{-1} in the brain of rats of 1 week of age and that this permeability is identical to that of adult rats. The vascular space occupied by mannitol (the initial volume of distribution V_i) falls from 1.23 ml . 100 g^{-1} at 1 week of age, to 0.75 ml . 100 g^{-1} brain in the adult rat (Preston et al., 1995), indicating either a larger vascular volume, resulting from a greater capillary density or capillary diameter in the neonatal rat, or a significantly greater degree of internalisation of the mannitol by the endothelium, possibly by fluid-phase endocytosis into the cerebral capillary endothelial cells, in the newborn, compared to the adult.

There is nothing to suggest that the human BBB is not at least as well formed at birth as it is in the rat. Occludin and claudin-5 expression is detected in the capillary endothelium of the brain of the 14 week human fetus and shows the same distribution at cell margins as seen in the adult (Virgintino et al., 2004). Pioneering studies by Grontoft (1954) in stillborn human fetuses from approximately 12 weeks gestation and from perinatal deaths have demonstrated a post-mortem BBB to trypan blue is present from at least the start of the second trimester, which is comparable to that of the adult human.

Blood–brain barrier in pathology

There is a growing list of CNS pathologies involving an element of BBB dysfunction, including multiple sclerosis (Correale and Villa, 2007); hypoxia and ischemia (Kaur and Ling, 2008); edema (Rosenberg and Yang, 2007); Parkinson's disease and Alzheimer's disease (Desai et al., 2007; Zlokovic, 2008); epilepsy (Remy and Beck, 2006); tumours (Bronger et al., 2005); glaucoma (Grieshaber and Flammer, 2007) and lysosomal storage diseases (Begley et al., 2008) (Table 5). The barrier dysfunction can range from mild and transient tight junction opening to chronic barrier breakdown (Förster, 2008), and changes in transport systems and enzymes can also occur. Microglial activation is increasingly recognised as an early sign of CNS inflammation, even in disorders not previously regarded as inflammatory. In most cases it is not possible to determine whether barrier compromise is causal in disease onset, but barrier disturbance can often be seen to contribute to and exacerbate

Table 5
CNS pathologies involving BBB dysfunction.

CNS pathology	BBB dysfunction	References
Stroke	Astrocytes secrete transforming growth factor-β (TGFβ), which downregulates brain capillary endothelial expression of fibrinolytic enzyme tissue plasminogen activator (tPA) and anticoagulant thrombomodulin (TM). Proteolysis of vascular basement membrane/matrix. Induction of aquaporin 4 (AQP4) mRNA and protein at BBB disruption. Treatment with arginine vasopressin V1 receptor antagonist reduced the increase in BBB permeability induced in stroke model.	Tran et al., 1999 Lo et al., 2003 Tomás-Camardiel et al., 2005 Vakili et al., 2005
Trauma	Bradykinin, a mediator of inflammation, is produced and stimulates production and release of interleukin-6 (IL-6) from astrocytes, which leads to opening of the BBB.	Schwaninger et al., 1999
Infectious or inflammatory processes	Examples include bacterial infections, meningitis, encephalitis and sepsis. The bacterial protein lipopolysaccharide (LPS) affects the permeability of BBB tight junctions. This is mediated by the production of free radicals, IL-6 and IL-1 β. Interferon-β prevents BBB disruption. Alterations in P-glycoprotein expression and activity in the BBB.	Gaillard et al., 2003 Veldhuis et al., 2003 Roberts and Goralski, 2008
Multiple sclerosis	Breakdown of the BBB. Tight junction abnormalities. Downregulation of laminin in the basement membrane. Selective loss of claudin3 (shown with antibody vs Cl 1/3) in experimental autoimmune encephalomyelitis.	Minagar and Alexander, 2003 McQuaid et al., 2009 Oki et al., 2004 Wolburg et al., 2003
HIV	BBB tight junction disruption.	Dallasta et al., 1999 Berger and Avison, 2004 Buckner et al., 2006
Alzheimer's disease	Decreased glucose transport, downregulation of glucose transporter GLUT1, altered agrin levels, upregulation of AQP4 expression. Accumulation of amyloid-β, a key neuropathological feature of Alzheimer's disease, by decreased levels of P-glycoprotein transporter expression. Altered cellular relations at the BBB, and changes in the basal lamina and amyloid-β clearance.	Berzin et al., 2000 Kalaria, 1999 Cirrito et al., 2005 Tai et al., 2009 Zlokovic, 2005 Bell and Zlokovic, 2009
Parkinson's disease	Dysfunction of the BBB by reduced efficacy of P-glycoprotein.	Kortekaas et al., 2005 Desai et al., 2007 Bartels et al., 2008
Epilepsy	Transient BBB opening in epileptogenic foci, and upregulated expression of P-glycoprotein and other drug efflux transporters in astrocytes and endothelium.	Abbott et al., 2002 Marroni et al., 2003 Lazarowski et al., 2007
Brain tumours	Breakdown of the BBB. Downregulation of tight junction protein claudin 3; redistribution of astrocyte AQP4 and Kir4.1 (inwardly rectifying K^+ channel).	Papadopoulos et al., 2004 Davies, 2002 Wolburg, 2006 Liebner et al., 2000 Warth et al., 2004
Pain	Inflammatory pain alters BBB tight junction protein expression and BBB permeability.	Huber et al., 2001 Willis and Davis, 2008
Glaucoma	Opening of the BBB, possibly through diffusion of endothelin-1 and matrix-metalloproteinase-9 into peri-capillary tissue.	Grieshaber and Flammer, 2007
Lysosomal storage diseases (LSD)	May show changes in BBB permeability, and/or transport, depending on specific LSD	Begley et al., 2008

developing pathology (Persidsky et al., 2006). It is important to develop better diagnostic methods to identify and locate sites of barrier disturbance, as early intervention offers the possibility of reducing long-term disease progression and disability (Abbott et al., 2006).

Blood–brain barrier regulation

It is increasingly recognised that the BBB is a dynamic system, capable of responding to local changes and requirements, and able to be regulated via a number of mechanisms and cell types, in both physiology and pathological conditions. Such regulation includes changes in tight junction function (Balda and Matter, 2009), and in expression and activity of many transporters and enzymes (Abbott et al., 2006; Dauchy et al., 2009). Regulation is an efficient means of matching the activities of the blood–brain barrier to the requirements of the brain, whether for protection from circulating agents, adjustment of nutrient supply, or modification to facilitate local repair. This emphasises again the key role played by the brain endothelium and its associated cells in the daily functions of the brain. As more is learned about BBB regulation, opportunities will emerge for targeting the brain endothelium to maintain health and to aid recovery from injury or infection, so that the BBB becomes integral to developing strategies for drug therapy to the brain (Abbott et al., 2006).

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