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Chapter 17:

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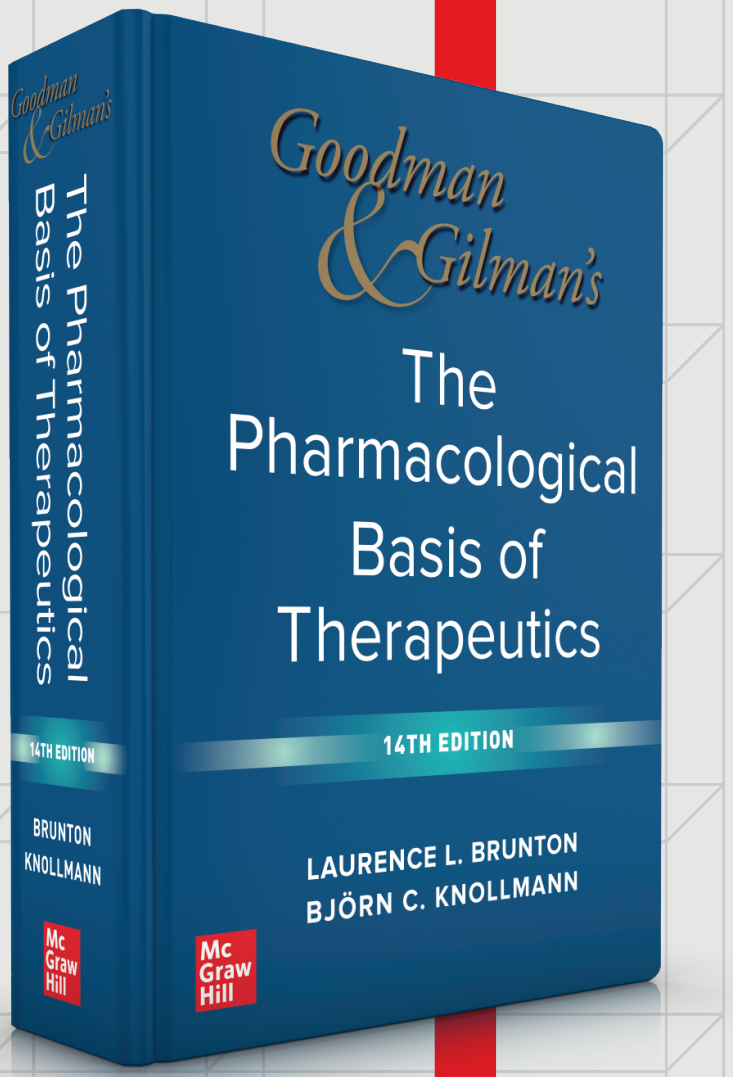

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Chapter 17 The Blood-Brain Barrier and Its Influence on Drug Transport to the Brain

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Brain Barriers

For a drug to be active, it must reach a certain concentration in the target tissue. The central nervous system (CNS) possesses a series of barriers that separate the neural tissue from the periphery. These barriers act to stringently regulate the movement of ions, molecules, and cells between peripheral fluids (i.e., blood) and the CNS, thus tightly regulating the extracellular environment of the CNS, which is critical to maintain homeostasis. The barriers not only control the influx of glucose and essential nutrients but also greatly limit the entry of many exogenous compounds, including drugs. The pharmaceutical industry has struggled with developing drugs that can cross these barriers and enter the brain without requiring high doses that give unwanted peripheral side effects or are too costly. For large-molecule drugs like antibodies, this problem is greater since larger molecules have an even lower ability to cross brain barriers.

The brain barriers include the blood vessels that vascularize the CNS parenchyma, the meningeal covering of the brain, and the choroid plexus within the ventricles (Figure 17–1).

The barriers are especially important to insulate the neurons from ionic fluctuations, such that the neurons can maintain appropriate ion gradients required for neural circuit function. The brain barriers also protect the CNS from toxins, pathogens, and even the body's own immune system, which is crucial because the CNS fails to regenerate after many injuries and diseases. The importance of the barriers is highlighted by the severe pathology of diseases in which the barriers are disrupted, such as multiple sclerosis, stroke, traumatic brain injury, meningitis, and other neurological injuries and disease. When intact, these barriers also create obstacles for drug delivery, as they greatly impede the entry of most blood-borne molecules from entering the brain.

The most studied barrier is the BBB, an endothelial barrier formed by the blood vessels that vascularize the parenchyma of the CNS. The BBB makes up most of the surface area of the brain barriers and hence is most important for drug delivery to the CNS. The meningeal coverings of the brain have two distinct barriers that restrict the movement of solutes from the periphery into the brain. The first barrier is the arachnoid barrier, which stringently regulates the passage of molecules and cells between the outer dura mater, which contains fenestrated "leaky" blood vessels, and the inner subarachnoid space, which contains the cerebrospinal fluid (CSF). This barrier is an epithelial barrier made up of arachnoid barrier cells that physically separate the dura mater and the subarachnoid space. The second barrier is an endothelial barrier possessed by the blood vessels within the subarachnoid space, which restrict the movement of molecules and cells between the blood and the CSF within the subarachnoid space. The choroid plexuses, which secrete CSF into the ventricles, also contain a barrier that is formed by choroid plexus epithelial cells that surround fenestrated "leaky" blood vessels within the plexuses. These epithelial cells act to tightly regulate the composition of the CSF that they are secreting into the ventricles, forming what is termed the blood-CSF barrier (BCSFB).

This chapter focuses on describing the cellular and molecular composition of the BBB, how the barrier properties are regulated, the role of the BBB in CNS pharmacology, and how the BBB is being targeted for CNS drug delivery.

The Blood-Brain Barrier

Cellular Composition of the BBB: The Neurovascular Unit

The BBB is a term used to describe the unique properties of the blood vessels that vascularize the CNS, which allows them to tightly regulate the movement of ion molecules and cells between the blood and the brain. Endothelial cells that form the walls of the blood vessels provide most the BBB properties, greatly restricting the passage of nonspecific molecules, while transporting specific nutrients into the CNS. Critical to the function of the BBB is also the interaction of the endothelial cells with other cells within the neurovascular unit including mural cells, fibroblasts, astrocytes, and immune cells (Figure 17–2).

Endothelial cells are thin cells that form the inner walls of all blood vessels, generating a lumen for blood to flow. In larger vessels, arteries and veins, the circumference of the vessel can be made up of dozens of endothelial cells, whereas the smallest capillaries can consist of a single endothelial cell folding upon itself to form a lumen of 6 to 8 μm in diameter.

Abbreviations

Apo: apolipoprotein
ATP: adenosine triphosphate
AUC: area under the concentration-time curve
BBB: blood-brain barrier
BCRP: breast cancer resistance protein
BCSFB: blood–cerebrospinal fluid barrier
CSF: cerebrospinal fluid
$f_{u,brain}$: unbound fraction of drug in brain homogenate
$f_{u,plasma}$: unbound fraction of drug in plasma
FUS: focused ultrasound
IR: insulin receptor
ISF: interstitial fluid
$K_{p,uu,brain}$: partition coefficient of unbound drug in brain interstitial fluid to that in plasma
$K_{p,uu,cell}$: partition coefficient of unbound drug between intracellular and interstitial fluids
LDLRf: low-density lipoprotein receptor family
MRP: multidrug resistance protein
PET: positron emission tomography
PS: permeability surface area product
RMT: receptor-mediated transcytosis
Tf: transferrin
TfR: transferrin receptor
$V_{u,brain}$: unbound volume of distribution in the brain; i.e., partitioning of total drug to that unbound in the brain interstitial fluid

The vascular network of the human brain is roughly 600 km long, with a thickness of 200 to 400 nm of the endothelial cells and a surface area of 15 to 25 square meters (Wong et al., 2013). Thus, the endothelial cells make up the primary cellular interface between the blood and the tissue and thus regulate permeability, transport, coagulation, and immune cell infiltration.

Mural cells are vascular support cells that sit on the abluminal surface of the endothelial cells, embedded in the vascular basement membrane, and regulate vascular parameters. On large vessels, *vascular smooth muscle cells* form concentric rings around the vessels and, through their contraction, control vessel diameter and thus blood flow. On capillaries and postcapillary venules, *pericytes* form incomplete layers, extending processes that interact with endothelial cells through peg-and-socket junctions. Pericytes regulate vascular permeability, endothelial immune activation, and blood flow (Armulik et al., 2011).

Fibroblasts are found embedded in the vascular smooth muscle layer of large vessels (Vanlandewijck et al., 2018b), sensing vascular stretch and regulating wound healing through the deposition of extracellular matrix.

A *basement membrane* consisting of extracellular matrix proteins including type IV collagens, laminins, nidogen, and heparan sulfate proteoglycans surrounds the CNS blood vessels. The basement membrane can be divided into two layers, the inner vascular basement membrane secreted by endothelial cells, mural cells, and fibroblasts, and the outer parenchymal basement membrane secreted primarily by astrocytes. These two basement membranes are separated in larger vessels but fuse around capillaries (Xu et al., 2019).

Astrocytes are a major glial cell population that extends polarized cellular processes; one set of processes ensheathes either synapses in the gray matter or nodes of Ranvier in the white matter, whereas the other process extends end feet that ensheathe more than 95% of the abluminal surface of the blood vessels, separated from the vessels by the basement membrane (Sofroniew and Vinters, 2010). Therefore, astrocytes are situated to sense and respond to both neural activity and vascular function and have been shown to regulate blood flow, vascular permeability, and CNS fluid dynamics as part of the glymphatic system (Hablitz and Nedergaard, 2021). In addition, neural progenitors, neurons, oligodendrocyte progenitors, and oligodendrocytes have been shown to interact with endothelial cells, regulating different aspects of vascular function.

Immune cells, both within the brain and within the blood, interact with CNS blood vessels. *Perivascular macrophages* are found in the perivascular spaces of draining venules where the glial lamina separates from the vascular basement membrane and survey this Virchow-Robin space as the first line of immunity in the CNS. *Microglia*, the CNS resident myeloid cells, extend highly motile ramified processes that survey the parenchyma and also poke between astrocyte end feet to inspect the vascular space (Li and Barres, 2018). These microglia have been shown to regulate vascular repair following injury (Lou et al., 2016).

Properties of the BBB

The BBB is not one entity, but a series of properties that allow the blood vessels to limit the passage of nonspecific molecules while delivering specific nutrients to the underlying neural tissue. Many of these properties are possessed by the endothelial cells that form the walls of the blood vesssels, such that CNS endothelial cells have distinct properties compared to endothelial cells in other tissues.

Glycocalyx

The *glycocalyx* is the carbohydrate-rich matrix that lines the luminal surface (blood side) of the endothelial cells and forms the first barrier to blood-borne molecules and cells. The vascular glycocalyx is made up of glycoproteins, proteoglycans, and glycolipids that can protrude several microns into the lumen of the vessel. Two-photon imaging in rodents has shown that this acts as the first barrier to large molecules, limiting their diffusion and ability to reach the endothelial cell (Kutuzov et al., 2018). It is currently unclear how the composition of the glycocalyx is different in CNS vessels compared to peripheral vessels and how the sieve-like barrier properties of the vascular glycocalyx are different in different tissues.

Tight Junctions

CNS endothelial cells are held together by *tight junctions* that form a tight paracellular barrier that polarizes the cell into distinct luminal and abluminal membrane compartments. Tight junctions, primarily studied in epithelial cells, are intercellular adhesions formed by transmembrane proteins including claudin family members, occludin, and junctional adhesion molecules that are linked to the cytoskeleton by adapters including zona occludens (Kniesel and Wolburg, 2000). The composition of claudins appears to determine the permeability of the paracellular pore, with claudin 5 being the most prominent claudin in CNS endothelial cells, creating a barrier that is greatly restrictive to ions with an electrical resistance of 1000 to 4000 ohms/cm^2. Genetic deletion of claudin 5 in mice results in a size-specific leakiness of the BBB to molecules less than 1000 Da (Nitta et al., 2003).

Transcellular Permeability

CNS endothelial cells restrict the transcellular movement of solutes with a *lack of fenestra* (pores in the membranes) and *low rates of transcytosis* (transcellular vesicle trafficking) compared to other vascular beds. Transcytosis in the endothelial cells can be divided into two mechanisms: (1) nonspecific transcytosis mediated through caveolin-coated vesicles and (2) receptor-mediated transcytosis of specific substrates through clathrin-coated vesicles. The tight paracellular barrier and low amounts of nonspecific transcytosis allow CNS endothelial cells to control the passage of molecules through transport. The receptor-mediated transport allows for the uptake of specific molecules including transferrin, insulin, and leptin (Yang et al., 2020).

Efflux Transport

The physical barrier properties restrict the passage of hydrophilic molecules across the endothelial cells; however, many small lipophilic molecules can passively diffuse across the endothelial cell membrane into the parenchyma. To regulate movement of lipophilic molecules across the BBB, CNS endothelial cells express a variety of *efflux transporters*

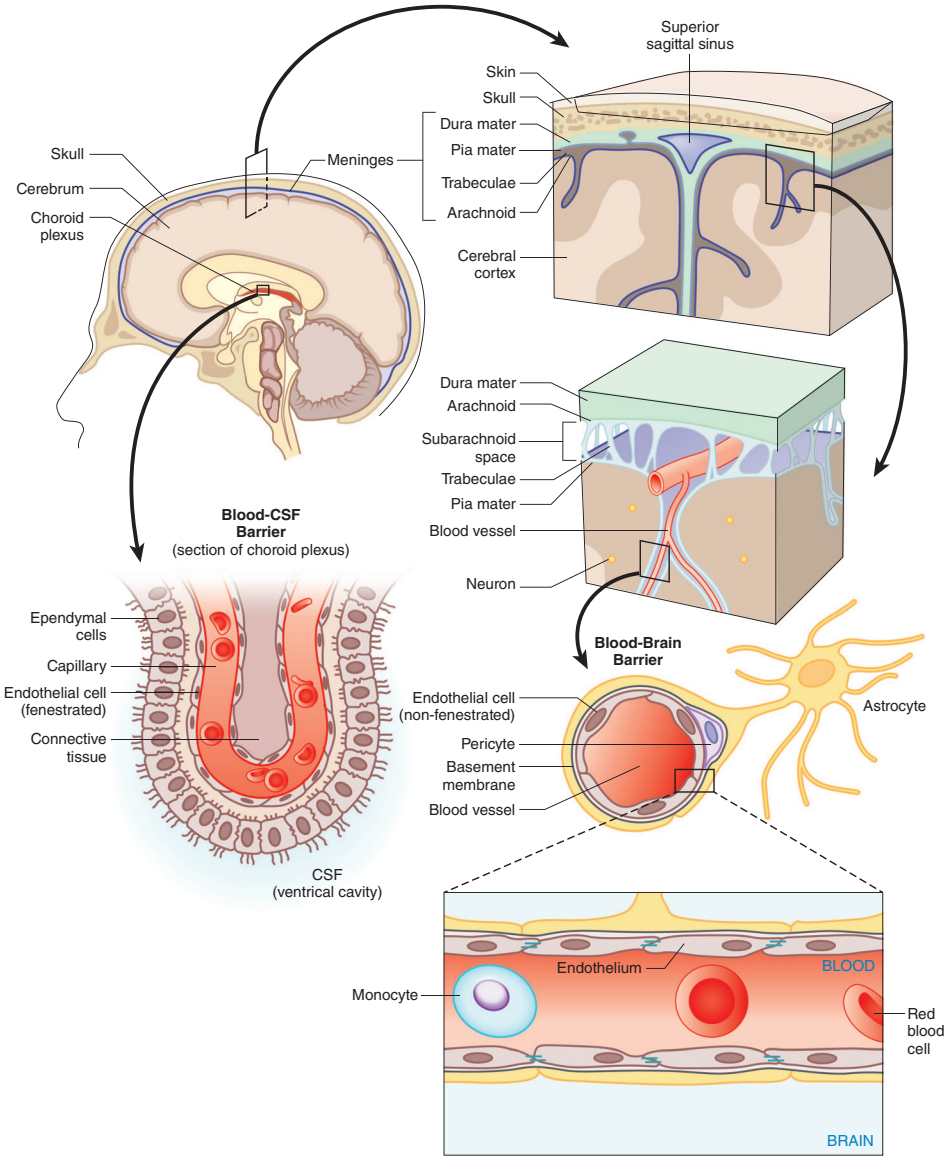

Figure 17–1 *Schematic representation of the major brain barriers.* Top right is a schematic of a cross-section through the meningeal coverings of the brain depicting the major meningeal barrier sites including the arachnoid barrier between the dura and the subarachnoid space and the vascular barrier possessed by the blood vessels within the subarachnoid space. Bottom left is a schematic of a cross-section through the choroid plexus that sits within the brain ventricles, depicting the choroid plexus epithelial cells that form the blood-CSF barrier between the leaky fenestrated vessels within the choroid plexus and the CSF within the ventricles. Bottom right depicts a cross-section of a parenchymal capillary that forms the BBB.

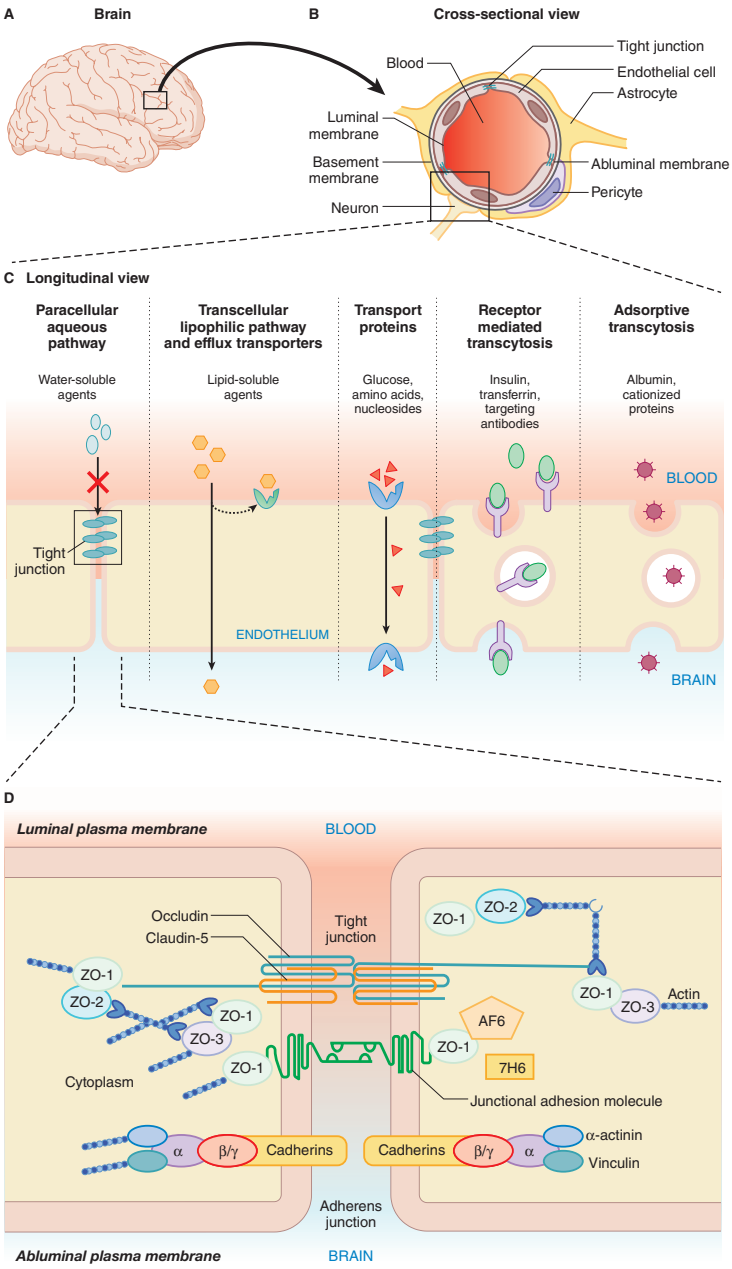

Figure 17–2 *Cellular and molecular anatomy of the BBB.* The vasculature of the brain (**A**) possesses specialized barrier properties that are induced and maintained by perivascular cells of the neurovascular unit (**B**). The BBB has a specialized network of transporters and properties to support selective transport of nutrients and metabolites into and out of the CNS (**C**). Brain endothelial cell adherens junctions and tight junctions are comprised of a complex network of cytoskeletal and transmembrane proteins (**D**).

including P-glycoprotein (ABCB1/MDR1) and breast cancer resistance protein (ABCG2/BRCP) (Loscher and Potschka, 2005) (Figure 17–2). These ABC transporters are present on the luminal membrane and use energy derived from the hydrolysis of adenosine triphosphate (ATP) to pump a wide array of substrates that would otherwise passively diffuse across the membrane, up their concentration gradient, and back into the blood. These transporters are critical modulators of drug delivery as they limit the entry of many small-molecule therapeutics into the brain.

Solute Transporters

The paracellular and transcellular barrier properties allow CNS endothelial cells to greatly restrict the movement of nonspecific molecules; however, the underlying neural tissue still requires specific nutrients for cell survival and physiological function. Therefore, CNS endothelial cells express a variety of *solute transporters* that facilitate the transport of specific substrates into the brain, including GLUT1 (glucose), LAT1 (amino acids), MCT1 (lactate), MCT8 (thyroid hormone), and others. Although peripheral tissues also require these nutrients, these transporters are largely lacking on the surface of peripheral vascular beds since these small molecules can easily access the tissues through paracellular routes. Lack of specific transporters in the BBB can lead to distinct neurological syndromes including De Vivo disease (GLUT1 deficiency) (De Vivo et al., 1991), an autistic syndrome (LAT1 deficiency) (Tarlungeanu et al., 2016), and psychomotor retardation (MCT8 deficiency) (Vatine et al., 2017). In addition, CNS endothelial cells express a variety of unique metabolic and signaling systems that act to regulate the extracellular composition of the CNS.

Immune Modulation

CNS endothelial cells also have much lower levels of *leukocyte adhesion molecules* compared to endothelial cells in other tissues. Binding of immune cells to leukocyte adhesion molecules is a critical step in their entry into a tissue. This is a multiple-step process that involves initial binding to the endothelium through selectins (e.g., E-selectin, P-selectin), rolling on the endothelium, firm adhesion through binding of immunoglobulin superfamily members (e.g., ICAM1, VCAM1), followed by transendothelial migration (see Figure 38–5). The low levels of these adhesion molecules on CNS endothelial cells correlate with very low amounts of immune cell infiltration across a healthy BBB, as most CNS immune surveillance occurs in CSF compartments including the ventricles and subarachnoid space. However, in many neuroinflammatory diseases, such as stroke, encephalitis, and multiple sclerosis, the endothelial cells can become activated and upregulate these adhesion molecules, leading to immune cell infiltration into the CNS. Inhibition of immune cell interactions with VCAM1 using *natalizumab*, an anti-VlA4 antibody, is effective at limiting new neuroinflammatory lesion formation in patients with relapsing-remitting multiple sclerosis (Polman et al., 2006).

Regulation of the BBB

To improve CNS drug delivery, it is critical to understand how the properties of the BBB are regulated within brain endothelial cells. Although many properties of the BBB are possessed by the endothelial cells, these properties can be induced and maintained by interactions with other cells within the neurovascular unit. This was first identified in transplantation studies, where blood vessels gained barrier properties upon vascularizing transplanted brain tissue, whereas brain blood vessels were found to lose barrier properties upon vascularizing the gut (Stewart and Wiley, 1981).

BBB Induction

Genetic manipulations in rodent models have identified that Wnt/β-catenin signaling drives CNS angiogenesis, but not angiogenesis in peripheral tissues, as well as the induction of tight junctions and solute transporter expression in the newly formed CNS vessels, indicating that specific barrier properties are induced as part of the CNS-specific angiogenic program (Daneman et al., 2009; Liebner et al., 2008; Stenman et al., 2008). Wnt/β-catenin signaling is also required for maintenance of the BBB, as genetic inhibition of this pathway in endothelial cells in adults leads to a cell-intrinsic reduction in tight junction proteins and leakage of blood vessels to non-specific molecules (Wang et al., 2012). The cellular source of Wnt signals appears to be neural stem cells and progenitors during the initial invasion of blood vessels into the CNS and other cells, including astrocytes and oligodendrocytes, later in development and through adulthood.

Pericytes and Astrocytes

Pericytes are also critical regulators of BBB properties, acting to limit the activated state of the endothelial cells. Genetic manipulations in mice that reduce pericyte coverage of CNS vessels lead to BBB leakage through an increase in nonspecific caveolin-mediated transcytosis as well as an increase in the expression of leukocyte adhesion molecules and thus the inflammatory state of the vessels (Armulik et al., 2010; Daneman et al., 2010). Astrocytes are also critical regulators of the barrier properties in endothelial cells: astrocyte-conditioned media can decrease paracellular permeability and increase endothelial efflux, and reactive astrocytes can regulate BBB repair following disease (Abbott et al., 2006; Bush et al., 1999). Therefore, BBB function is induced and maintained through a series of complex and coordinated cellular signals derived from neural progenitors, astrocytes and pericytes.

Heterogeneity of the BBB

Although the BBB is largely a property of the capillaries in the CNS, barrier properties are present throughout the vascular trees from the invading arterioles to the draining venules. It is critical that each branch possesses barrier properties, as leakage at any level of the vascular tree would disrupt CNS homeostasis. There is both molecular and functional heterogeneity along the different segments of the vascular tree with regulation of blood flow most prominent in the arterioles, transport properties most prominent in the capillaries, and immune cell interactions most prominent in the postcapillary venules (Aird, 2007a, 2007b; Vanlandewijck et al., 2018a).

In addition, there is regional heterogeneity of the BBB to differentially control the local environment of different brain regions. For example, the area postrema, the subfornical organ, the vascular organ of lamina terminalis, the median eminence, the pituitary neural lobe, and the pineal gland, together termed the circumventricular organs, lack BBB properties. Instead, the fenestrated vessels in these regions allow for diffusion of molecules between the blood and the neural tissue, which is critical to the neurosensory and neurosecretory function of these brain regions (Kaur and Ling, 2017). Tanycytes are cells that create borders around these regions providing a cellular barrier restricting the blood-derived molecules from entering neighboring brain regions. Less is known about whether there is heterogeneity of the BBB in brain regions that contain a functional BBB to meet the unique requirements of different brain regions. Interestingly, there are 6-fold differences in the uptake of *paliperidone*, a strong P-glycoprotein substrate, among different brain regions, indicating that regional heterogeneity of the BBB may be a critical modulator of drug uptake (Loryan et al., 2016).

Plasticity of the BBB in Health

Blood-brain barrier function is not static but can change at different stages of life and in response to different stimuli. During embryonic development, the earliest invading vessels have immature junctions, large amounts of transcytosis, and high levels of leukocyte adhesion molecules, and thus are leakier than mature blood vessels (Siegenthaler et al., 2013). In aging, there is a decrease in specific receptor-mediated transcytosis and an increase in nonspecific caveolin-mediated transcytosis; thus, the BBB less stringently regulates the extracellular environment of aged brains (Yang et al., 2020). The properties of the BBB can also be dynamically regulated by signals coming from both the brain and periphery. For example, neural activity can decrease barrier efflux (Pulido et al., 2020), likely coordinating detoxification of the brain during rest periods with other brain exit routes including the glymphatic system. Furthermore, diet has been shown to alter the components of brain endothelial cell tight junctions, thus modulating the paracellular permeability (Salameh et al., 2019). Hence, the BBB is not simply a static wall that protects that brain, but a dynamic component of the neural circuitry, changing in response to different stimuli. These are important principles for understanding how the BBB regulates brain homeostasis and acts as an obstacle for drug delivery.

BBB in Disease

Blood-brain barrier dysfunction is observed in many neurological diseases, including stroke, multiple sclerosis, traumatic brain injury, epilepsy, and other neuroinflammatory and neurodegenerative diseases. This dysfunction can involve an increase in transcytosis, a loss of tight junction integrity, alterations in transporter expression, and an increase in the inflammatory state of the endothelial cells, which can lead to massive leakage of the BBB (Profaci et al., 2020). This leakage is often localized spatially to the region insult and temporally to specific pathophysiological stages of the injury and disease. For instance, in patients with multiple sclerosis, there is a leakage of the BBB, as measured by gadolinium enhancement on magnetic resonance imaging, at the location of neuroinflammatory lesions specifically at the onset of lesion formation. This leakage can be harnessed to target drug delivery to specific injured regions of the CNS within the specific time window of leakage (Miller et al., 1988).

Drug Delivery Across the BBB

Given the unique properties the BBB possesses, drug delivery across the BBB is a challenge. A circulating drug first encounters the endothelial glycocalyx, which reduces drug penetration by binding and sequestering molecules as they diffuse to the endothelial cell surface. If a drug molecule can penetrate the glycocalyx, it next needs to cross the barrier-forming endothelial cells of the BBB. There are several possible modalities for the trans-BBB passage of drug molecules (see Figure 17–2).

1. *Paracellular transport by diffusion between adjacent endothelial cells:* This process works for water-soluble molecules in peripheral vascular beds but is negligible at the BBB because of the high resistance tight junctions. Indeed, the introduction of small hydrophilic tracers into the bloodstream is often used as a measure of BBB integrity given their extremely low brain penetration. As a result of this physical barrier, drug penetration through the endothelial tight junctions does not result in therapeutic concentrations in the brain.
2. *Crossing plasma membranes:* Drug molecules can diffuse serially through the endothelial cell plasma membranes. For this process, drugs need to be small (<500–1200 Da) and rather lipophilic. In many cases, however, drugs having more lipophilic physiochemical properties are substrates for the efflux transporters P-glycoprotein, breast cancer resistance protein (BCRP), or members of the multidrug resistance protein (MRP) family. Thus, despite the cell-penetrable physiochemical properties, lipophilic drugs may not reach the brain because the active barrier of efflux transporters pumps drugs back to the bloodstream.
3. *Co-opting nutrient transporters:* For drugs that are large and/or hydrophilic, other delivery routes must be identified. One strategy takes advantage of the nutrient transporters expressed at the BBB. If a drug molecule is structurally similar to a nutrient, it may be possible for it to transport across the BBB using the endogenous transporter. For instance, the prodrug *levodopa* is structurally similar to phenylalanine, allowing it to enter brain endothelial cells via the large neutral amino acid transporter, after which it is subsequently metabolically converted to dopamine (Wade and Katzman, 1975). Unfortunately, steric limitations on such membrane carriers prevent their use with many drug cargoes. It is also important to note that since brain endothelial cells are polarized, the influx and efflux transporters can be found with differential abundance on the blood and brain side endothelial membranes, and this polarization may also impact the efficiency of trans-BBB delivery.
4. *Co-opting receptor-mediated transporters:* The endosomal trafficking network of brain endothelial cells can be targeted for drug delivery. Nonspecific fluid-phase endocytosis or pinocytosis and subsequent transcytosis are very limited at the BBB in healthy conditions, so drug uptake solely through this pathway is insufficient. However, the endosomal trafficking network of brain endothelial cells can be co-opted with carefully designed drug delivery constructs. For instance, protein cationization can lead to interactions with the brain endothelial cell surface and trigger the process known as adsorptive-mediated transcytosis. Alternatively, endothelial cell surface receptors that interact with endogenous large-molecule ligands like transferrin can be targeted using a variety of strategies, allowing conjugated drug molecules to cross the BBB and enter the brain.
5. *BBB opening:* Another option for crossing the BBB involves chemical or physical disruption of the tight junctions such that paracellular diffusion through the spaces between endothelial cells is enhanced. While this can increase drug uptake into the brain, it is inherently nonspecific in that other blood components can also enter the brain upon BBB disruption, potentially resulting in detrimental side effects.

Diffusion Into Brain

Once a drug leaves the brain endothelial cell, it also encounters the vascular basement membrane that surrounds endothelial cells and their associated pericytes and the glial limitans, an additional barrier formed by the extracellular matrix proteins secreted by astrocyte foot processes that wrap the brain vasculature. To reach neuronal or glial therapeutic targets, drugs must therefore diffuse through both the basal lamina and glial limitans before they can diffuse through the brain extracellular space. The brain extracellular space is estimated to have an average pore size of about 40 to 60 nm (Thorne and Nicholson, 2006), which could limit the transport of antibodies and nanoparticulate therapeutics.

Taken together, several routes exist for drug entry into the brain. The delivery of small-molecule therapeutics to the CNS by crossing plasma membranes is by far the most clinically advanced approach. With the continued development of protein and gene medicines (biologics) that are too large to transport through the endothelial cell membranes, there have also been significant recent efforts in the co-opting of receptor-mediated transport systems and leveraging of BBB opening to deliver biologics. The principles governing small-molecule transport into the brain are discussed below, followed by parallel efforts in developing brain delivery paradigms for biologics.

Small Molecules

When a drug is administered, independent of being given orally, intravenously, subcutaneously, or intramuscularly, it will first distribute into the bloodstream and from there out in the whole body. In all tissues and organs, there is further a distribution across the capillaries into the tissue and further into cells. As the brain constitutes around 2% of whole-body weight, most of the administered drug will distribute to the rest of the body, although clinically relevant concentrations can be obtained in the brain.

To enter the brain, the drug must cross the BBB. It is only the unbound drug molecules that can transverse the BBB. The molecules that are bound to plasma proteins cannot. Once inside the brain, it is only the unbound molecules that can interact with the target. Thus, what is nonspecifically bound to tissue components or to plasma proteins in the blood can be considered as an inactive reservoir in rapid equilibrium with the unbound, freely moving molecules. Therefore, measuring the unbound concentration is the most important way to understand membrane transport and drug action, without confounding the measurement with binding in plasma or to tissue components.

Drug distribution can be considered as a series of equilibria across barriers and between bound and unbound moieties of the drug. There is also an equilibrium between unionized and ionized drugs that is dependent on the pK_a of the drug and the pH at the specific site. In Figure 17–3, the equilibria relevant for drug transport across the BBB are depicted.

Rate and Extent of Transport Across the BBB

Transport across the BBB can have different rates depending on the drug's physicochemical properties in relation to those of the membrane. Also, different relationships can exist between the unbound concentrations in brain interstitial fluid (ISF) and plasma, due to the efflux and uptake transporter's efficiency. Thus, the rate and the extent of transport are two important aspects of transport across the BBB. These are two independent properties governing the pharmacodynamics of drug action in the brain. For anesthesia before surgery, drugs with rapid transport are sought after. It is also optimal that the anesthesia goes away quickly after surgery. For

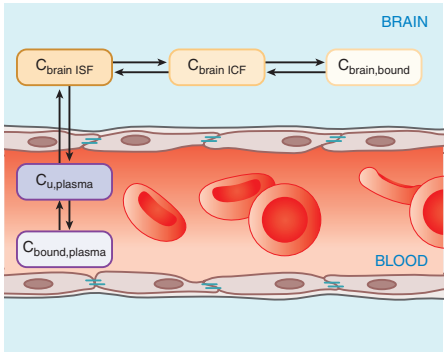

Figure 17–3 *Drug transport and equilibration across the BBB and into the brain parenchyma.* Drugs distribute in blood plasma between what is free and what is bound to plasma proteins. Only the free, unbound molecules can transverse the endothelial cell layer of the brain capillaries and reach the interstitial fluid (ISF) and go back into blood. Once inside the brain parenchyma, the drug equilibrates into cell intracellular fluid (ICF) and binds to cellular components. C describes the concentration.

other drugs that are taken daily for chronic diseases, the rate is not as important as the extent (i.e., how much drug enters the brain). Instead, we want high enough concentrations in the brain for action over time. Therefore, even though the transport across the BBB may be slow, a drug may still enter in enough quantities to be valuable. The extent of transport is therefore the most relevant property for drugs given chronically.

From Figure 17–3, it is clear that if a drug has a constant concentration in plasma and the passage across the BBB is passive (i.e., not influenced by any transporters either hindering or helping uptake into the brain [influx clearance = efflux clearance]), the unbound concentrations will be the same on both sides of the BBB at steady state. If transporters at the BBB hinder uptake (influx clearance < efflux clearance), the concentration in brain ISF will never reach as high levels as those in plasma. Conversely, if transporters in the BBB pick up drug from plasma (influx clearance > efflux clearance), the brain ISF concentration will become higher than those in plasma. This is illustrated in Figure 17–4. The ratio between brain ISF and unbound plasma concentrations is named $K_{p,uu,brain}$ (the

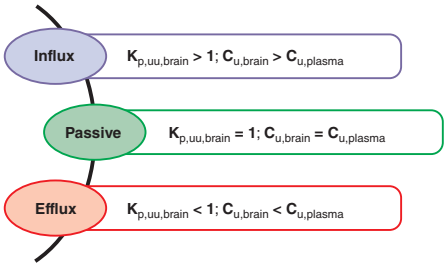

Figure 17–4 *Drugs equilibrate across the BBB in three ways depending on the activity of transporters, as described with $K_{p,uu,brain}$, the partition coefficient of drug concentrations across the BBB between unbound in brain ISF and unbound in plasma at steady state.* When efflux transporters like P-glycoprotein hinder transport into the brain, the unbound brain ISF concentration ($C_{u,brain}$) will never reach the same concentration as that in plasma ($C_{u,plasma}$) and $K_{p,uu,brain}$ will be lower than unity. When drug can transverse the BBB freely, the unbound concentrations will be similar in brain ISF and plasma, and when there are active uptake transporters acting on the drug, the concentration in brain ISF will be higher than that in plasma, and $K_{p,uu,brain}$ will be above unity.

partition coefficient of unbound drug across the BBB or, expressed differently, the concentration ratio of unbound drug between brain ISF and plasma) (Gupta et al., 2006; Hammarlund-Udenaes et al., 2008). Depending on the efficiency of an efflux transporter to hinder uptake, the ratio can be somewhat or much smaller than unity. The most potent efflux transporter for small molecular drugs is P-glycoprotein. Conversely, if a drug is actively taken up into the brain at the BBB, the higher the efficiency of uptake transport, the higher is the brain ISF concentration. Glucose enters the brain using the glucose uptake transporter GLUT1, but as glucose is rapidly consumed in the brain, the concentration is not generally higher than in plasma. Drugs are normally not metabolized in brain tissue. Therefore, their transport properties will more directly influence their concentration in the brain versus that in plasma.

$K_{p,uu,brain}$ can be expressed as a concentration ratio and as a ratio of clearances across the BBB:

$$K_{p,uu,brain} = \frac{C_{u,brain}}{C_{u,plasma}} = \frac{CL_{in}}{CL_{out}} \qquad \text{(Equation 17–1)}$$

where CL_{in} is the net influx clearance at the BBB from plasma to brain ISF and CL_{out} is the net efflux clearance from brain ISF to plasma (see Figure 17–4). The unit for clearance is µL/min/g brain.

In general, many more drugs are effluxed at the BBB than taken up actively. Examples of drugs on the market and their $K_{p,uu,brain}$ values are depicted in Figure 17–5. Measuring $K_{p,uu,brain}$ is so far done only in preclinical studies (Loryan et al., 2014). The values given in Figure 17–5 are therefore mainly from rat studies. Humans have a less "efficient" P-glycoprotein function at the BBB; thus, drug delivery to the brain may be higher in humans than in rodents (Syvanen et al., 2009; Uchida et al., 2020).

$K_{p,uu,brain}$ values of drugs provide important information for determining drug action in the CNS. For therapeutic targets in the CNS, drug developers should aim at higher $K_{p,uu,brain}$ values. To minimize CNS side effects, it should be low. At the same time, drug potency needs to be considered.

$K_{p,uu,brain}$ values can vary drastically within a drug class and can determine their therapeutic actions. Opioids are a good example: *Loperamide* is a strong P-glycoprotein substrate that is very efficiently effluxed. Its $K_{p,uu,brain}$ is less than 0.01, indicating that less than 1% equilibrates across the BBB. In other words, more than 99% is kept out of the brain by the BBB. On the other hand, *morphine* is only mildly effluxed out of the brain ($K_{p,uu,brain}$ = 0.3), and *oxycodone* is actively taken up at the BBB ($K_{p,uu,brain}$ = 3), reaching 3-fold higher concentration in the brain than plasma. Hence, the BBB actions determine the clinical use of these opioids: *Loperamide* can be used for diarrhea without effects from the CNS, while *morphine* and *oxycodone* are centrally acting analgesics. *Oxycodone* also shows much faster uptake into the brain than *morphine* (Bostrom et al., 2006, 2008). Although *morphine* and *oxycodone* have a 10-fold difference in their extent of BBB transport, they are both active in the CNS, as other pharmacokinetic factors are also contributing, including the dose that is used to give a clinically relevant effect.

Another class of drugs with varying brain delivery is the antihistamines. The newer generation antihistamines, like *cetirizine* or *loratadine*, are all significantly effluxed, thereby causing much less central side effects such as sedation. On the other hand, *diphenhydramine* is actively taken up at the BBB with a $K_{p,uu,brain}$ of 5 (i.e., five times higher concentrations in brain ISF than unbound in blood), explaining why it causes much more sedation. *Diphenhydramine* in fact has the highest active brain uptake of all drugs measured thus far.

Intrabrain Distribution

After traversing the BBB, the drug enters the brain parenchyma and distributes in the ISF and cells (Figure 17–6). The concentration in brain ISF is the driving force for further distribution. The distribution can involve passive diffusion and binding as well as active uptake into or efflux from cells. Intrabrain distribution can be described as the ratio of total amount of drug in the brain parenchyma to unbound drug concentration in brain

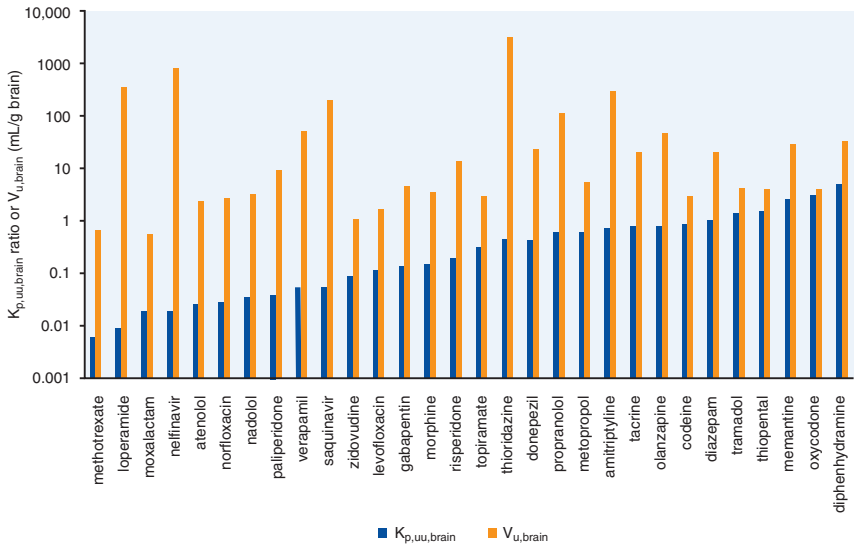

Figure 17–5 *Examples of how different drugs partition across the BBB and how they distribute in the brain parenchyma, showing that the two properties are not at all related.* This is described by the partition coefficient of unbound drug in brain ISF to that in plasma $K_{p,uu,brain}$ (blue) and the intrabrain distribution parameter $V_{u,brain}$ (orange), the unbound volume of distribution of drug in the brain, estimated as the ratio of the total amount of drug per gram of brain divided by the unbound concentration in brain ISF (mL/g) (describing both binding and/or intracellular distribution).

ISF, also called $V_{u,brain}$ (the unbound volume of distribution in the brain, expressed in mL/g brain). Approximating that 1 g brain equals 1 mL, this can be considered as a ratio, where the unbound volume of distribution in the brain $V_{u,brain}$ (mL/g brain) is described as:

$$V_{u,brain} = \frac{A_{tot,brain} - V_{blood\ in\ brain} \cdot C_{tot,blood}}{C_{u,brainISF}} \qquad \text{(Equation 17–2)}$$

$A_{tot,brain}$ is the total amount of drug in the brain per gram of brain. As there is also blood in the brain capillaries, this amount needs to be subtracted from the total amount in the brain to obtain a correct value. Therefore, $V_{blood\ in\ brain}$ is the physiological volume of blood in the brain, and $C_{tot,blood}$ is the total concentration of drug in the blood present in the brain. The blood volume in brain is 3% or less, depending on the sampling technique. $C_{u,brainISF}$ is the unbound concentration of drug in the brain ISF.

The values found so far of $V_{u,brain}$ range from 0.6 mL/g brain for *moxalactam* to 3300 mL/g brain for *thioridazine* (see Figure 17–5). The higher the value, the more the drug is bound to or distributed into cells than what is present in brain ISF. Some drugs, generally the more lipophilic ones, distribute and bind extensively to brain tissue components, as the brain parenchyma is also lipophilic in nature. Thus, BBB transport and brain drug binding are two independent parameters. A drug can have a very low BBB transport but quite extensive distribution and binding in the brain parenchyma (see Figure 17–5). The opposite is also possible (i.e., a drug can have a rather high BBB transport but not so extensive binding in brain). For example, the antiarrhythmic agent *verapamil* has a $K_{p,uu,brain}$ of 0.05 and a $V_{u,brain}$ of 54 mL/g brain, the antiviral drug *nelfinavir* has a $K_{p,uu,brain}$ of 0.02 and a $V_{u,brain}$ of 860 mL/g brain, while *diazepam* has a $K_{p,uu,brain}$ of unity, thus being mainly passively transported, and a $V_{u,brain}$ of 20 mL/g brain (i.e., 20 times more bound and/or distributed than unbound in brain). Drugs that do not enter cells to any significant degree have $V_{u,brain}$ values below unity. This is the case for *methotrexate* due to its high hydrophilicity.

Thus, when only total brain concentrations are measured, a candidate for further development may erroneously be selected that has high total brain concentrations but much lower unbound concentrations. Thus, if the high concentration is due to high binding, the part that is active (i.e., the unbound part) is much lower. Over the last decades, this has resulted in selection of many unsuccessful candidate drugs.

Intracellular Distribution

Drugs are taken up into cells due to pH partitioning as well as active uptake or efflux from the brain parenchymal cells (see Figure 17–6). In this context, cells of different types are averaged to a "typical" cell, independent of whether they are neuronal cells or glial cells. Of course, the distribution into these different cell types may differ, something that can be studied in cell cultures. The purpose here is to obtain an overview of how drugs are generally distributed.

Due to a lower intracellular pH, especially in lysosomes and other acidic organelles, basic drugs tend to accumulate in brain parenchymal cells, whereas acidic drugs do not. Basic drugs therefore tend to cause more side effects due to their accumulation in lysosomes, so-called lysosomotropism.

The ratio of unbound drug between intracellular and interstitial fluids is called $K_{p,uu,cell}$ (see Equation 17–3). As for the BBB, a ratio around unity indicates mainly passive transport, values below unity indicate reduced uptake into cells, and values above unity indicate accumulation into the brain parenchymal cell.

$$V_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,brainISF}} \qquad \text{(Equation 17–3)}$$

where $C_{u,cell}$ is the average unbound concentration in cells and $C_{u,brainISF}$ is the concentration in brain ISF.

The equilibration of drug between the brain ISF and the average brain intracellular compartment, $K_{p,uu,cell}$, is a separate property from the overall binding and distribution to brain parenchyma ($V_{u,brain}$) (Figure 17–7).

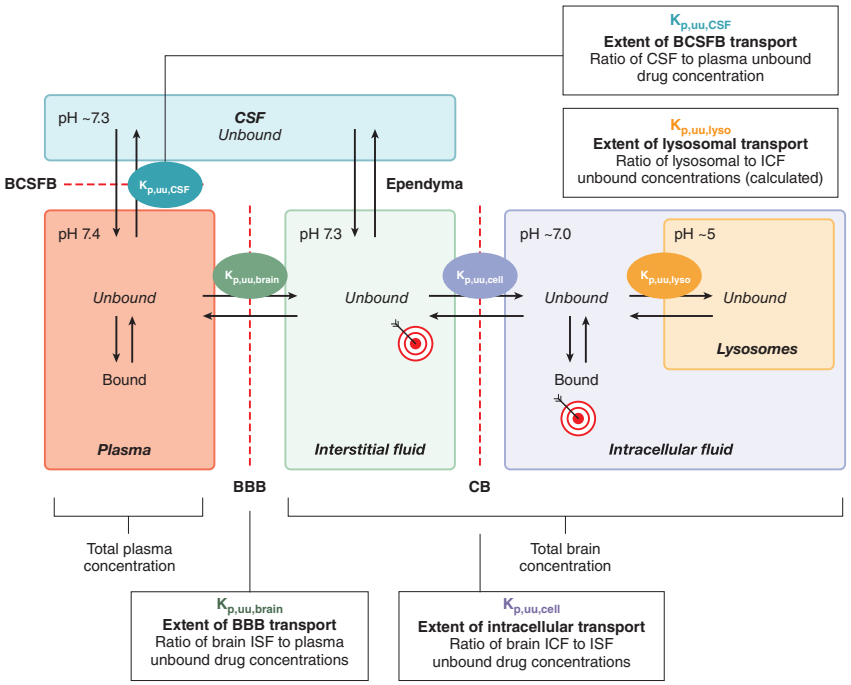

Figure 17–6 *Schematic representation of the equilibration of drug across the brain and cellular barriers (CB) and their representative parameters.* To be noted is the pH difference between the different compartments, influencing basic and acidic drugs to distribute differently, with basic drugs tending to accumulate into acidic lysosomes. ICF, intracellular fluid.

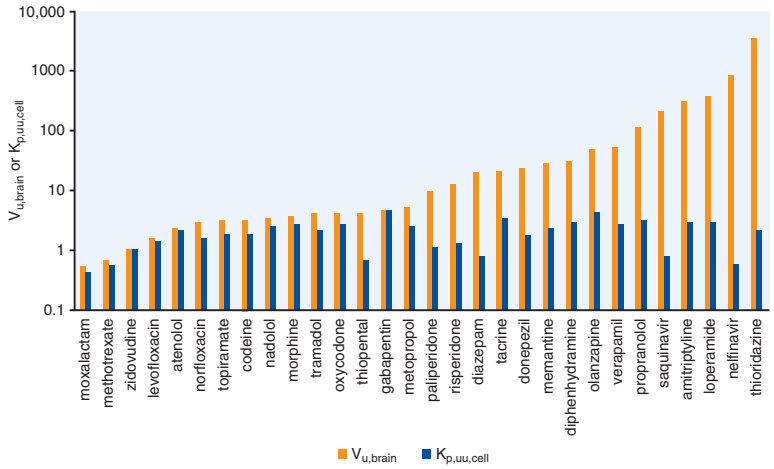

Figure 17–7 *Examples of how drugs distribute and bind in the brain parenchyma on average ($V_{u,brain}$) and their cellular partitioning ($K_{p,uu,cell}$), showing no relationship between the two.* This indicates that they are independent properties, where some drugs are accumulating into cells ($K_{p,uu,cell} > 1$) and others are not ($K_{p,uu,cell} = 1$ or < 1).

The binding is more governed by lipophilicity, while the cellular distribution, as described with $K_{p,uu,cell}$, is governed by other processes including pH partitioning. For example, compared to *methotrexate* with a $K_{p,uu,cell}$ of 0.6, the antidepressant *amitriptyline* with a $K_{p,uu,cell}$ of 2.9 accumulates intracellularly to much higher levels (Figure 17–7). A special case is *gabapentin*, which does not bind to cellular components, as shown by a $V_{u,brain}$ of close to unity, but exhibits a $K_{p,uu,cell}$ of 5. The likely explanation is the presence of an active uptake mechanism for *gabapentin* at the cellular barrier.

Whether drug concentrations in a given brain compartment are relevant for drug effects is dependent on the potency of the drug together with where the target is situated, be it toward brain ISF or cytosol or in the nucleus (see Figure 17–6). The same goes for side effects, as mentioned earlier with lysosomotropism.

CSF Versus Brain ISF Concentrations of Drugs

Cerebrospinal fluid is used as a surrogate site for measuring unbound drug brain concentrations in humans, as there is no other way of measuring unbound brain concentrations directly. Lumbar puncture is used to collect CSF. CSF has very little protein in healthy individuals, meaning that CSF drug concentrations are largely unbound. However, in disease, the protein concentration in CSF may increase. To understand the relationship of drug concentrations in CSF versus brain ISF, anatomical and physiological comparison of CSF versus brain ISF production and relation to plasma concentrations of the drugs must be considered. As discussed earlier, CSF is mainly produced at the BCSFB, in the choroid plexus (see Figure 17–1), while the main origin of brain ISF comes from the BBB. Transporter expression and hence active transport at the BBB are somewhat different than at the BCSFB. Thus, CSF drug concentrations may differ from those in brain ISF. For example, the transporter MRP2 is present at the BCSFB but not at the BBB (Gazzin et al., 2008). Nevertheless, the few studies that have been performed *in vivo* indicate that CSF concentrations are a reasonable estimate of ISF concentrations for most drugs (Friden et al., 2009).

Drug Interactions at the BBB

Drug-drug interactions could be expected since the transporter activity is high at the BBB. One might envision that two drugs that are both substrates for P-glycoprotein may compete with each other, resulting in higher brain concentrations of both drugs. However, to date, no such interaction has been reported. A likely explanation is that the plasma concentrations that the BBB "sees" are quite low in relation to the capacity of the transporters to handle the drug transport (i.e., the transport is not saturated). This contrasts to the liver and the gastrointestinal tract, where often much higher drug concentrations are present that can saturate transport systems (see Chapters 2 and 5 for details). In that regard, *in vitro* cell studies using much higher concentrations than those attained *in vivo* may suggest potential drug-drug interactions that are not observed *in vivo*.

Methods to Study BBB Transport

To measure BBB transport of drugs, the gold standard is microdialysis, where a microdialysis catheter is placed in a specific brain region and concentrations there are compared with unbound plasma concentrations (Chaurasia et al., 2007). Unfortunately, the method cannot be used for many drugs that bind avidly to plastic material in the catheter. Useful for those drugs is a combination of several methods, called the "combinatory mapping approach" (Loryan et al., 2014). Here, the total brain and plasma concentrations are measured at steady state, combined with plasma protein binding and brain tissue binding with the brain slice and brain homogenate techniques. These three measurements together will give the $K_{p,uu,brain}$:

$$K_{p,uu,brain} = \frac{C_{tot,brain}}{C_{tot,plasma} \cdot V_{u,brain} \cdot f_{u,plasma}} \qquad \text{(Equation 17–4)}$$

$C_{tot,brain}$ and $C_{tot,plasma}$ are the corresponding total concentrations at steady state, and $f_{u,plasma}$ is the unbound fraction of drug in plasma, measured with equilibrium dialysis of plasma versus buffer at pH 7.4. $V_{u,brain}$ is measured with the brain slice technique (Loryan et al., 2013), alternatively as a surrogate with the brain homogenate method, where $f_{u,brain}$ (unbound fraction of drug in brain homogenate) ≈ $1/V_{u,brain}$. However, the brain homogenate lacks pH partitioning and active membrane transport due to the homogenization of the tissue cells, which reduce the quality of the measurement.

To measure intrabrain distribution, the brain slice and the brain homogenate methods can be combined, resulting in $K_{p,uu,cell}$ (Equation 17–5). If the pK_a of a drug is known, it is also possible to estimate the partitioning between lysosomal and intracellular compartments, resulting in $K_{p,uu,lyso}$ (Figure 17–6).

$$K_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,brain\ ISF}} = V_{u,brain} \cdot f_{u,brain} \qquad \text{(Equation 17–5)}$$

Positron emission tomography (PET) is a noninvasive method that can measure brain concentrations in humans. However, it measures total radioactivity and thereby both bound and unbound drug, as well as metabolites. Ongoing research attempts to translate PET measurements to unbound drug concentrations, which would give much more information on how humans are handling drugs at the BBB (Gustafsson et al., 2019).

Biologics

The BBB properties of tight junctions and limited pinocytosis severely restrict the uptake of large-molecule biologics into the brain. For example, only 0.03% to 0.1% of monoclonal antibodies administered intravenously reach the brain (Jones and Shusta, 2009), and thus, therapeutic concentrations are rarely achievable even under high dosing regimens (e.g., 20–50 mg/kg). Hence, biologics such as protein, DNA, and nanoparticles must use alternative routes for brain entry. Such routes include targeting the endosomal trafficking network of brain endothelial cells via adsorptive or receptor-mediated transcytosis where appropriately targeted drug cargo can engage with an endothelial cell receptor and piggyback across the BBB into the brain. For adsorptive or receptor-mediated processes, the steps for crossing the BBB include (1) binding to the endothelial cell surface via nonspecific charge interactions (adsorptive) or by targeting a specific BBB receptor (receptor-mediated); (2) inducing the formation and endocytosis of vesicles; (3) trafficking through the endothelial cell; (4) exocytosis; and (5) therapeutic release at the basolateral membrane (Figure 17–8). Alternatively, pathological BBB disruption or BBB disruption through chemical or physical means can be leveraged to increase brain entry of blood-borne biologics.

Quantifying Brain Uptake of Biologics

The critical measurement to understand potential therapeutic efficacy is the drug concentration that can be achieved in brain tissue. Initial rate pharmacokinetic analyses can allow the comparison of biologic delivery strategies as they relate to BBB permeability, the property that ultimately determines drug concentrations reached in brain. As is the situation for biologics, where the BBB permeability is relatively low compared with blood flow (Bickel, 2005), initial rate pharmacokinetics indicate that the brain uptake (%ID/g) is a function of the BBB permeability surface area (PS) product and the area under the plasma concentration curve (AUC):

$$\%\frac{ID}{g} = PS \times AUC \qquad \text{(Equation 17–6)}$$

where %ID/g is the percentage of the injected dose delivered to brain per gram of brain, PS is the BBB permeability surface area product (μL/min•g), and AUC is the area under the plasma concentration curve (%ID•min/μL).

As seen from Equation 17–6, the brain uptake will be directly proportional to the BBB permeability of the biologic through the PS product.

Thus, the PS product is instructive for comparing various strategies for crossing the BBB. The PS product is a lumped parameter describing the entire BBB transport process from binding to the brain endothelial cell to release into the brain (steps 1–5 in Figure 17–8). Importantly, the PS products for different targeting approaches and transport systems are not equivalent due to differences in receptor density and the percentage of internalized receptors that undergo transendothelial transport rather than recycling or being targeted to the lysosome (see Figure 17–8). In addition, brain uptake depends on the dose and systemic clearance properties of the molecule, quantified as the area under the brain concentration curve (AUC). Importantly, the AUC can be influenced by the brain specificity of the biologic targeting strategy. An ideal biologic delivery system would combine high trans-BBB transport that approaches or exceeds the natural ligand (Table 17–1) with high brain specificity and reduced clearance

TABLE 17–1 ■ TRANSPORT OF ANTIBODIES AND NATURAL LIGANDS AT THE BBB

	PS PRODUCT [mL/g-s × 10^6] (% ID/brain)	TRANSPORTER (MECHANISM)	ANIMAL	REFERENCE
Human IgG	0.062 (ND)		Rat	[1]
Albumin[a]	0.097 {0.062}[b] (ND)		Rat	[1]
Tf[a]	2.432 {0.062}[b] (ND)	TfR (RMT)	Rat	[1]
Insulin[a]	18.50 {0.062}[b] (ND)	IR (RMT)	Rat	[1]
Cationized IgG	9.5 (ND)	NS (AMT)	Rat	[2]
Cationized D146 MAb	ND (0.07) {0}	NS (AMT)	Mouse	[3]
MAb OX26	27 (0.44) {0}[c]	Rat TfR (RMT)	Rat	[4], [5]
MAb OX26	0.77 (0.03)[c]	Rat TfR (RMT)	Mouse	[6]
Mab RI7-127	20 (0.8)[c]	Mouse TfR (RMT)	Mouse	[6]
MAb 8D3	25 (1.5)[c]	Mouse TfR (RMT)	Mouse	[6]
MAb 128.1	ND (0.3) {0.06}	Human TfR (RMT)	Monkey	[7]
MAb Z35.2	ND (0.2) {0.03}	Human TfR (RMT)	Monkey	[7]
MAb 83-14	88–90 (2.5–3.8) {0.06}	HIR (RMT)	Monkey	[8, 9]
Chimeric MAb 83-14	28 (2)	HIR (RMT)	Monkey	[9]
Humanized MAb 83-14	ND (1)	HIR (RMT)	Monkey	[10]

MAb, monoclonal antibody; ND, not determined or not available in reference; NS, nonspecific; Tf, transferrin; TfR, transferrin receptor; HIR, human insulin receptor; RMT, receptor-mediated transcytosis; AMT, adsorptive-mediated transcytosis.

[a]Average PS product from various brain regions, determined in referenced article.

[b]Numbers in {} are values for isotype control antibodies and are indicators of general antibody permeability.

[c]Values were calculated from reported %ID/g, assuming representative mass of animal brain (100 g for monkey, 1 g for rat, and 0.5 g for mouse).

1. Poduslo JF, et al. Macromolecular permeability across the blood-nerve and blood-brain barriers. *Proc Natl Acad Sci USA*, **1994**, *91*:5705–5709.
2. Triguero D, et al. Cationization of immunoglobulin G results in enhanced organ uptake of the protein after intravenous administration in rats and primate. *J Pharmacol Exp Ther*, **1991**, *258*:186–192.
3. Pardridge WM, et al. Enhanced cellular uptake and in vivo biodistribution of a monoclonal antibody following cationization. *J Pharm Sci*, **1995**, *84*:943–948.
4. Bickel U, et al. Delivery of peptides and proteins through the blood-brain barrier. *Adv Drug Deliv Rev*, **1993**, *10*:205–245.
5. Friden PM, et al. Antitransferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. *Proc Natl Acad Sci USA*, **1991**, *88*:4771–4775.
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7. Friden PM, et al. Characterization, receptor mapping and blood-brain barrier transcytosis of antibodies to the human transferrin receptor. *J Pharmacol Exp Ther*, **1996**, *278*:1491–1498.
8. Pardridge WM, et al. Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries in vitro and rapid transctosis through the blood-brain barrier. *Pharm Res*, **1995**, *12*:807–816.
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(increased AUC). Thus, throughout the sections below, PS product and AUC are used as means for comparison of different strategies for biologic delivery to brain.

Confirming Brain Exposure of Biologics

Although kinetic approaches are accurate measures for brain delivery, they are based on whole-brain uptake assays that include both the vasculature and the brain components. Hence, any biologic that is bound to or internalized into brain endothelial cells (e.g., Figure 17–8) but not yet transported fully into brain will be included in these measures. Importantly, when using transcytosis-based delivery approaches, the fraction of delivered therapeutic that can be "trapped" in the vasculature and not available for brain exposure can be substantial. Several measurement methods have been introduced to circumvent this challenge.

A method known as capillary depletion has been developed to separate the vascular component from the parenchymal component. Since the BBB is surrounded by a robust basement membrane, it is possible to separate the microvasculature from brain using mechanical homogenization and density centrifugation techniques. In this way, antibody that remains associated with the vasculature can be distinguished from that which has transcytosed fully into the parenchymal fraction. However, homogenization techniques can lead to a large fraction of negative control antibodies appearing to accumulate in the parenchymal fraction. To help circumvent this issue, parenchymal uptake time course measurements can be performed (Pardridge et al., 1991).

Immunocytochemical techniques have been used to qualitatively demonstrate parenchymal uptake of biologics into brain at microscopic resolution. However, when a biologic leaves the concentrated trafficking vesicles in endothelial cells and enters brain, it undergoes a roughly 1000-fold dilution. Thus, unless the biologic also subsequently targets a receptor on brain cells that allows it to reconcentrate, it often escapes detection. Autoradiographic techniques can be more sensitive and allow for semiquantitative uptake analyses, but at much lower resolution, such that distinguishing between vascular and parenchymal contributions can be difficult. The final strategy for measuring the quantity of biologic that transcytoses and enters brain is to monitor its accumulation in the CSF (Haqqani et al., 2013). While sampling of CSF has long been used as a surrogate measure of brain uptake, one has to distinguish the BBB and choroid plexus routes of entry into the CSF to avoid overestimating the amount of biologic crossing the BBB and leading to brain exposure.

Researchers have also developed pharmacodynamic approaches to quantify brain uptake of biologics that rely on the functional output of the biologic. For example, putative transcytosing antibodies have been connected to drug cargo that allows for straightforward phenotypic readouts in animal models. A widely used model involves the conjugation of a BBB-crossing antibody with an antiamyloid therapeutic; a decrease in the amyloid burden in a transgenic mouse is used as a surrogate measure of full BBB crossing of the therapeutic at pharmacologically relevant concentrations. Another strategy is based on delivery of the neuropeptide neurotensin. Neurotensin causes transient hypothermia, changes in locomotion, and changes in pain response if directly administered into the CNS, whereas blood-borne neurotensin cannot cross the BBB to elicit these effects. However, if neurotensin is conjugated to a BBB-crossing domain, it can accumulate in the CNS, causing pharmacodynamic responses, again demonstrating full transcytosis of the biologic at pharmacologically relevant doses (Demeule et al., 2014).

Adsorptive-Mediated Strategies

One approach for delivery of biologics is to modify the molecules themselves such that they can access the adsorptive-mediated transport network of brain endothelial cells. Cationization of a biologic can allow for its interaction with negatively charged moieties on the endothelial surface such as sialylated proteins and heparan sulfate proteoglycans. Such engagement can lead to membrane invagination and entry into the endosomal trafficking system of brain endothelial cells. In this way, biologics can transport into and across brain endothelial cells, leading to increased brain uptake. Cationization is often achieved by modifying carboxyl residues on the protein surface, such as through the conjugation of putrescine or spermine (Poduslo and Curran, 1996). To rate the efficiency of various BBB-crossing approaches for biologics, the resulting PS product can be compared. For instance, cationized antibodies had 4- to 100-fold higher PS products compared with control antibodies (see Table 17–1). While cationization can increase brain uptake, cationization will also increase interactions with vascular beds and cells throughout the body, leading to off-target drug accumulation. In addition, broad organ uptake can also reduce the AUC of circulating biologics, thereby limiting brain uptake (%ID/g) despite improved PS attributes.

Receptor-Mediated Strategies

Since adsorptive strategies are inherently nonspecific, alternative strategies have been developed that target specific receptor-mediated transcytosis (RMT) receptors at the BBB. Brain endothelial cells express a host of RMT receptors that bring large-molecule cargo into the brain. These include the transferrin receptor, insulin receptor, and low-density lipoprotein family receptors. Conceptually, RMT systems can be co-opted for brain drug delivery by using a natural or artificial targeting ligand conjugated to the drug of interest. The drug-ligand conjugate can bind to RMT receptors on the blood side of the brain endothelial cells and trigger endocytosis and trafficking, and a subset of the trafficked material can undergo full transcytosis. In this way, conjugated drug cargo can be shuttled across the BBB and into brain (see Figure 17–8). The transport properties of the targeting ligands can be further optimized through the modulation of targeting ligand binding affinity and the number of binding sites per ligand (valency). Several RMT-based strategies have recently moved to the clinic but are not yet at the point of FDA approval, as will be described below.

Transferrin Receptor

The transferrin receptor (TfR) was the first RMT system explored for delivery of biologics across the BBB (Pardridge et al., 1991), and it is still the most commonly used system. The TfR is highly abundant in the brain vasculature. TfR is responsible for transporting iron into the brain by mediating the trafficking of the iron-binding protein transferrin (Tf). The TfR has been targeted by conjugating therapeutic cargo to Tf itself. For example, Tf has been conjugated to monoclonal antibodies and various forms of nanoparticulate cargo, including pegylated albumin nanoparticles and pegylated liposomes, with the potential to increase brain uptake severalfold. Alternative strategies have employed iron-mimicking peptides that bind to Tf and ferry across the BBB with conjugated drug cargo. Targeting RMT receptors with the natural ligand-drug conjugate has a substantial drawback because to achieve transport of the desired drug conjugate, it needs to compete successfully with the endogenous natural ligands. For example, endogenous Tf is present at high concentrations in the bloodstream; hence, any Tf-drug conjugates will need to compete with this Tf pool for RMT binding and transport, necessitating high doses. Thus, research teams have instead been developing antibodies capable of targeting RMT receptors using epitopes that do not overlap with the natural ligand. In this way, the antibodies will not compete with endogenous ligand, which can help with delivery efficiency and potentially reduce side effects by not interfering with normal nutrient transport. Such antibodies raised against the TfR have elevated PS products by 20- to 30-fold that meet or exceed those of the natural ligand, leading to %ID in the 0.5% to 2% range (see Table 17–1). Targeted antibody strategies yield PS products that can exceed those of cationized antibodies by severalfold. Brain exposure of anti-TfR antibodies can reach actual brain concentrations as high as 50 nM with antibody optimization and elevated doses of 20 to 50 mg/kg (Yu et al., 2011). As such, TfR antibodies have been used to deliver a wide range of therapeutic cargo to animal models (e.g., delivery of antiamyloid antibodies in mouse and primate models of Alzheimer's disease). Anti-TfR–based biologics have already moved into clinical development. For example, conjugates of anti-TfR

antibodies with lysosomal enzymes are being tested in patients with lysosomal storage diseases like Hunter syndrome with early evidence of efficacy (Okuyama et al., 2019) (JCR Pharmaceuticals NCT04573023 and Denali NCT04251026).

Insulin Receptor

The insulin receptor (IR) has also been targeted for biologics delivery to brain. The IR at the BBB mediates brain import of insulin by an RMT mechanism. Targeting this transport system using the endogenous insulin ligand has not been pursued owing to the very short serum half-life of insulin and concerns that insulin-linked drugs could cause hypoglycemia. Thus, monoclonal antibodies against IR have been tested for their potential as RMT-targeting agents. The most widely used anti-IR antibody is a mouse antibody known as 83-14 that targets the human IR (Pardridge et al., 1995). This antibody has a PS product that is 4-fold higher than the native insulin ligand, suggesting it is an efficient BBB-targeting moiety. Uptake in primate brain can reach nearly 4%ID. For clinical translation, the 83-14 antibody has been humanized to help prevent unwanted immunogenicity as a result of the murine antibody framework. As with the TfR, conjugation of therapeutic cargo with the 83-14 antibody can lead to increased brain uptake. For example, in primates, pegylated liposomes carrying transgene-encoding DNA could lead to selective expression of transgene within targeted neuronal populations. Also, 83-14 conjugation with neurotrophic cargo led to 10-fold increases in brain levels. Moreover, when the lysosomal enzyme that is deficient in patients with mucopolysaccharidosis type I is conjugated to 83-14, it could also enter primate brain. In a clinical trial, the humanized 83-14 antibody-enzyme construct stabilized cognitive function in patients with severe forms of mucopolysaccharidosis (Giugliani et al., 2018) (Armagen NCT03053089, NCT03071341).

The therapeutic drug cargo needs to be carefully mated to the antibody-RMT system for successful deployment. Given that the total concentrations of antibody uptake in brain remains in the 1 to 50 nM range even under high dosages, the conjugated therapeutic needs to be efficacious at these concentrations. For this reason, early clinical trials with anti-TfR and anti-IR antibodies have focused on delivery of enzymes that catalytically process many substrate molecules and, hence, operate within the modest concentration ranges afforded by targeted RMT systems.

Low-Density Lipoprotein Family Receptors

Low-density lipoprotein receptor family (LDLRf) receptors such as LRP1, LRP2, and LDLR are expressed at the BBB. These RMT receptors mediate the transport of lipoproteins and other ligands across the BBB. They may have significant potential for trans-BBB transport as the brain uptake rates of LDLRf ligands such as receptor-associated protein and melanotransferrin (P97) exceed that observed for Tf, suggesting a high-capacity RMT pathway (Demeule et al., 2002; Pan et al., 2004). Only ligand-based LDLRf approaches have been described thus far. Apolipoprotein (Apo) B and ApoE are protein constituents of lipoproteins that mediate interactions with LDLRf receptors. Fusion of therapeutic cargo to ApoB and ApoE receptor binding domains can lead to brain uptake and pharmacological effects in rodent models including the delivery of an Aβ-degrading enzyme and lysosomal enzymes. Similarly, a peptide based on a conserved binding motif of several LDLRf ligands known as the Kunitz protease inhibitor domain has been used for trans-BBB delivery. Known as Angiopep-2, this peptide enters the brain via LRP1 RMT receptor, and when conjugated to neurotensin, it can elicit pharmacodynamic responses. Subsequently Angiopep-2 has used to deliver genes, peptides, and small-molecule P-glycoprotein substrates to the brain. Angiopep-2 was tested in phase I and II clinical trials for the delivery of *paclitaxel* for primary and metastatic brain tumors (e.g., Angiochem, NCT00539383, NCT01967810). These trials have demonstrated brain uptake of the conjugate and have suggested therapeutic efficacy (Kurzrock et al., 2012).

Improving BBB Transport of RMT-Targeted Systems

Transferrin receptor, IR, and LDLRf RMT systems have significant drawbacks despite early success in clinical studies. They target RMT systems that are not exclusive in their BBB expression, but rather are expressed all throughout the body both at the vascular and tissue levels. For molecules targeting these RMT systems, this leads to a decreased plasma AUC because of peripheral organ uptake, which in turn can lead to a decrease in brain uptake. Moreover, side effects can be caused by conjugated drug cargo accumulation in nonbrain tissues. Identification of other RMT systems with more brain specificity would be desirable. In addition, the commonly used RMT systems may not have the optimal capacity for trans-BBB delivery either because of receptor abundance or differential trafficking dynamics. For instance, antibodies targeting the TfR can be sequestered and degraded within the brain endothelial cells, limiting full transcytosis across the BBB. As such, the PS products for all RMT systems are not necessarily the same. Therefore, to increase the brain uptake of RMT-targeted biologics, one needs to increase the AUC or PS products of the targeting molecules. Two strategies for improving these parameters are to search for more BBB-specific RMT systems, which could increase the AUC due to less uptake in the periphery, or to optimize the current antibody-RMT systems by increasing their BBB PS product. Hence, ongoing research is focused on finding better BBB RMT systems and optimization of existing antibody-RMT systems.

BBB Opening

The BBB can be disrupted by pathological conditions such as stroke and brain cancer, but the timing and extent of disruption are often not adequate for efficient therapeutic delivery. On the other hand, chemical and physical methods can enhance the BBB opening and have been employed for drug delivery to the brain.

Chemical Methods

Chemical methods include the use of intra-arterial infusion of hyperosmolar *mannitol* that can transiently and reversibly disrupt the BBB in one brain hemisphere by opening the tight junctions in brain endothelial cells. *Mannitol* disruption has been deployed in the clinic for the treatment of glioblastoma and used in combination with therapeutic antibodies and small molecules (e.g., *bevacizumab*, NCT00968240; *cetuximab*, NCT02861898; and *temozolomide*, NCT01180816) and may enhance chemotherapy delivery, although the efficacy needs to be further evaluated (Neuwelt et al., 1983). Unfortunately, the method is inherently nonspecific and causes blood vessel opening throughout the entire targeted brain hemisphere and not just in the diseased region. The mechanism of opening allows not only the therapeutic to enter, but also any other blood-borne substances, which can lead to significant side effects such as seizures and brain edema. The patient also needs to be anesthetized for the procedure.

To identify agents that could be more selective in their regional modulation of BBB permeability, several biochemical agents have been used. These include ATP-sensitive and calcium-activated potassium channel activators that appear to selectively increase tumor BBB permeability by increasing the number of transport vesicles at the tumor BBB. Another approach is to activate bradykinin type 2 receptors. Bradykinin receptor activation can selectively increase tumor BBB permeability without breakdown of the healthy BBB. The mechanisms driving BBB opening are complex and may include both tight junction disruption and increased nonspecific transcytosis. In clinical studies, bradykinin analogues increased chemotherapeutic uptake into glioma tissue (Emerich et al., 2001). Region-specific BBB modulation with chemical approaches usually relies on the selective expression of brain endothelial targets in the diseased region. Hence, treatment paradigms aimed at preferentially increasing BBB permeability in the diseased brain region tend to be disease specific and are not generalizable.

Physical Methods

Physical methods, unlike biochemical methods, can be targeted to specific brain regions regardless of pathology. The most advanced physical BBB opening approach is focused ultrasound (FUS) (Figure 17–9). Microbubbles administered systemically are excited by focused ultrasonic

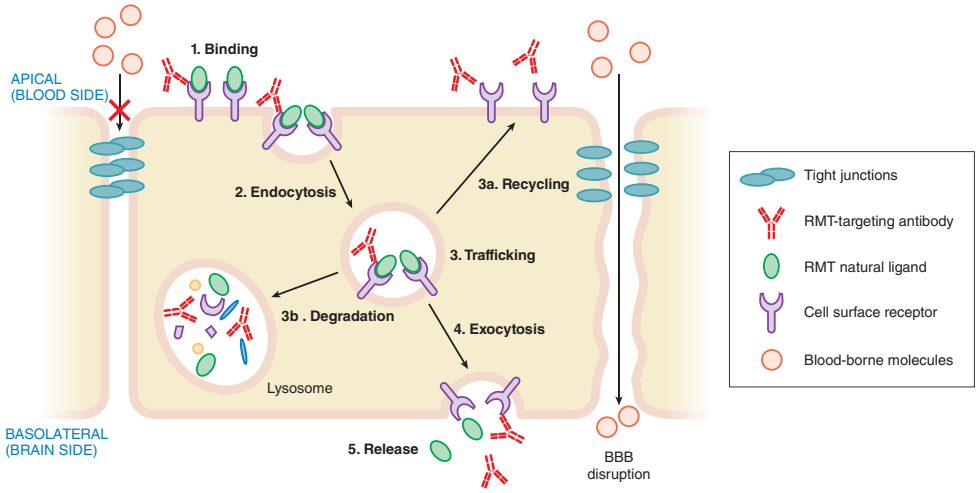

Figure 17–8 *Schematic representation of the BBB RMT mechanism.* A natural ligand or antibody targeting an RMT receptor traffics through the brain endothelial cell vesicle transport network. Transcytosis across the BBB and into brain would constitute a molecule passing through steps 1 to 5.

waves, which in turn transiently and reversibly disrupt the BBB in FUS-treated regions.

The FUS procedure generates mechanical shear, which is thought to downregulate tight junction proteins and upregulate transcellular transport machinery, thereby increasing BBB permeability. After FUS, uptake of small molecules and biologics is increased in targeted regions. As with chemical methods, FUS-mediated BBB opening is not selective, and blood-borne substances can enter brain tissue, causing unwanted side effects. The mechanical shear forces may also cause immune activation and hemorrhage. FUS is being evaluated in several clinical trials for patients with glioma, neuropathic pain, Parkinson's and Alzheimer's disease (glioma, NCT03322813; neuropathic pain, NCT03309813; Parkinson's disease, NCT02347254; and Alzheimer's disease, NCT03671889). BBB-opening methods are increasingly studied for enhancing therapeutic uptake in the CNS. Given potential side effects, they may be best suited for conditions that do not require chronic treatment and repeated opening of the BBB.

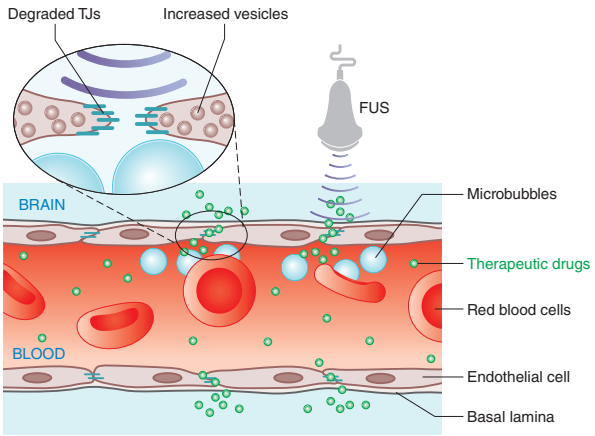

Figure 17–9 *Schematic representation of BBB disruption by focused ultrasound (FUS).* Upon FUS application, microbubbles apply mechanical forces on brain endothelial cells leading to increased transport vesicles and disrupted tight junctions (TJs).

Summary

The BBB poses specific hurdles for brain disease drug development. Different components of the neurovascular unit cooperate to maintain the BBB in brain endothelial cells. The BBB is necessary for healthy brain function but challenges drug delivery. Some small nonpolar molecular drugs easily cross the BBB, but many drugs do not, due to the presence of active efflux transporters at the BBB. On the other hand, efflux transporters can minimize CNS side effects, such as for antihistamine sedation. Some drugs co-opt the active uptake systems that are expressed at the BBB. The function of the BBB limits the entry of antibodies and other biologics even more severely. Strategies to circumvent the BBB using adsorptive-mediated or receptor-mediated transcytosis are currently in development, and chemical or mechanical BBB disruption are additional possibilities for enhancing brain drug uptake.

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