Physiology of the blood–brain barrier and its consequences for drug transport to the brain

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Abstract. The brain endothelium forms the blood–brain barrier (BBB) and is the chief site regulating molecular traffic between blood and brain. The ‘barrier phenotype’ includes tight junctions restricting paracellular flux and a range of transport mechanisms controlling transcellular flux. The barrier is induced by cell types associated with the microvessels and is subject to regulation. The composition of the BBB membranes will influence the permeability of lipid-soluble compounds. Specific transporter systems for solute uptake are present on both the apical (luminal) and basal (abluminal) membranes and efflux transporters of broader specificity are also present. Certain larger molecules may cross via transcytotic vesicular mechanisms. Potential drug molecules designed to enter the brain may use or interact with one or more of these routes—better understanding is needed before it will be possible to establish the optimal means of delivery for specific compounds and to design or modify them accordingly. Many lipid-soluble drugs are substrates for efflux transporters, making it difficult to guarantee CNS delivery or calculate free concentration in the brain interstitial fluid (ISF). The dynamics of the flowing ISF also complicate modelling and prediction of CNS pharmacokinetics. Finally, many disease states involve BBB dysfunction, which needs to be taken into account in designing appropriate therapies. © 2005 Published by Elsevier B.V.

Keywords: Blood–brain barrier; Drug delivery; Interstitial fluid; Permeability; Transport

1. Introduction

The brain is the most delicate organ in the body. It is the control centre for a range of critical bodily functions from respiration to feeding and is essential for all the normal
activities of daily life. In humans, the majority of neurons that will form the adult brain are present from the time of birth or soon after, with very limited capacity for cell division thereafter [1]. The network of neural connections controlling normal activities is also established early in life. It is hence essential that conditions within the brain permit neuronal longevity.

Higher order neural activity requires precise homeostasis of the neural microenvironment, efficient delivery of nutrients and removal of waste products, protection from neuroactive and toxic substances circulating in the blood, and maintenance and repair processes that take into account the inability of the majority of neurons to divide and replicate. Sites where blood comes into contact with neural tissue are critical interfaces for all these functions and at these sites barrier mechanisms have evolved, important in creating the optimal environment for neural physiology [2]. Improved understanding of neuropathological and neurodegenerative conditions now offers real prospects of targeted drug therapy to the brain, but efficient and intelligent design of appropriate drugs will require detailed knowledge of the mechanisms regulating trafficking of molecules including drugs at CNS barrier sites. The blood–brain barrier (BBB) at the level of brain microvessels creates the largest surface area ‘barrier interface’ (12–20 m²/1.3 kg brain) and has the greatest influence on drug delivery to the brain. This review focuses on the physiology of the BBB and its consequences for drug permeability and transport to the brain.

2. The multi-tasking BBB

Neural signalling requires precise homeostatic regulation of the interstitial fluid (ISF) of the brain. Three ‘barrier’ sites contribute to this regulation: the blood–brain barrier (BBB) formed by the brain endothelial cells lining cerebral capillaries, the arachnoid membrane of the meninges, and the choroid plexus epithelium that secretes cerebrospinal fluid (CSF) [3]. Of these, the BBB is the most important site for regulating drug access to the brain, given its large surface area and the short diffusion distances from capillaries to neurons (typically <10–15 μm [4]). The BBB also supplies key nutrients and protects the brain from neuroactive and potentially toxic compounds circulating in the plasma. The barrier function of the BBB is a combination of physical restriction (tight junctions reduce paracellular permeability for hydrophilic molecules), transport regulation (uptake and efflux carriers and selective transcytosis regulate transcellular molecular flux) and metabolic activity (enzymes metabolise many potentially harmful agents) [5,6]. This ‘multi-tasking’ BBB has played a major role in the evolution of the brain as a complex and integrated neural network [2] but poses problems for therapeutic approaches that require the delivery of drugs and other molecules to the brain for the treatment of CNS disorders [7].

3. Cell types at the blood–brain barrier

The main physical barrier is formed by the capillary endothelial cells lining the microvessels, which are coupled by much tighter junctions (zonulae occludentes) than found in peripheral vessels [8]. The endothelial cells secrete and are surrounded by a basal lamina (BL), with the end-feet of astrocytic glial cells closely apposed to its opposite side.
In places, pericytes are embedded in the BL between endothelial cell and astrocyte, making particularly close (invaginating) contacts with the endothelial cells. A number of other cell types may be present in the perivascular space, including perivascular macrophages (from the blood monocyte lineage) [6], and in larger vessels (arterioles, arteries, veins), smooth muscle occupies the position of pericytes within an expanded collagenous extracellular matrix. Microglia are the resident macrophages of the nervous system and may also adopt a perivascular location; terminals from a number of neuronal populations may end on or near the vessels, or show varicosities characteristic of ‘en passant’ transmitter release sites. There is increasing evidence that the community of cells at the BBB works in a closely integrated way and together with the neurons supplied by the segment of the microvessel forms the ‘neurovascular unit’ [9], a functional module servicing the metabolic and other requirements of the neuronal population. Close associations between individual astrocytes and endothelial cells may form a subcomponent of this, the ‘gliovascular unit’ [10]. Moreover, many of the key features characteristic of the ‘BBB phenotype’ appear to be induced in the brain endothelium via chemical influences from the associated cell types [11].

4. Physiology of the blood–brain barrier

4.1. The physical barrier: tight junctional structure

The tightness of the BBB appears not only from the physical complexity of its junctional structure (number of strands of transmembrane particles visible in freeze-fracture images, making an anastomising and dense network [12]) but also from the molecular substructure, in particular presence of transmembrane proteins claudins 1 and 5 that help to seal the intercellular cleft and impose selectivity [8]. The claudins function within a complex scaffold of other transmembrane (occludin) and cytoskeletal proteins (including coupling proteins of the ZO family, ZO 1–3) linked to the actin cytoskeleton, with adherens junctions providing additional structural strength. The net result is a strong and restricting cell:cell connecting zone that runs around the endothelial cell margin like a zipper, severely restricting penetration of polar (hydrophilic) molecules through the cleft, the ‘paracellular pathway’. This forces molecular traffic to take a largely transcellular route across the brain endothelium [7].

4.2. The nature of the endothelial cell membrane: influence on passive drug permeability

Like all cells, the brain endothelium possesses an outer cell membrane (plasmalemma) composed of a lipid bilayer with embedded proteins, some of them membrane-spanning. Small gaseous molecules such as oxygen and carbon dioxide can diffuse freely through the membrane, enabling oxidative metabolism of the brain and facilitating its pH regulation. The majority of drugs used to treat the CNS are lipid-soluble (lipophilic) and able to diffuse through the endothelial membranes [13]. Lipid analysis of the brain endothelium shows a high cholesterol content (~20% w/w), with the main phospholipids being phosphatidylethanolamine (~30%) and phosphatidylcholine (~20%) [14]. Artificial membrane systems designed to mimic BBB lipids and act as a useful high throughput screening tool for new drugs need to have a composition similar to this (and different from GI equivalents) in order to accurately reproduce the rank order of
CNS drug permeability [15]. Examination of the physicochemical profiles of 405 CNS drugs showed that although there were apparent average optima for molecular weight and lipophilicity (MW ~280, LogP<sub>octanol</sub> ~2.0), these differed according to primary therapeutic category [13], which is likely to reflect the need not only to partition in and penetrate BBB membranes but also to show activity at the target site of action, often a more polar site (e.g. receptor, transporter). However, this kind of study treats the membrane as a homogeneous and unchanging unit and it is likely that improved understanding of the microorganisation of membrane domains (rafts, molecular clusters [16]) and its relation to the cytoskeleton will lead to more sophisticated prediction and ranking of passive membrane permeability of CNS drugs.

4.3. Uptake transporters

In peripheral tissues, most of the nutrients and waste products that require molecular exchange with the blood are able to move via the intercellular cleft—the junctions here are sufficiently tight to restrict penetration of large molecules such as plasma proteins but permeable to small hydrophilic solutes. However, the paracellular pathway for small molecule traffic between blood and brain is severely restricted by comparison with the peripheral vasculature; where traffic is needed, it must be handled by specific transport systems in the endothelial membranes. More than 20 such carriers/transporters for uptake into the brain endothelium (uptake transporters) have been identified for glucose, amino acids, nucleosides, nucleobases, and a number of organic anions and cations [6].

Many BBB transporters, such as the GLUT-1 glucose carrier, are equilibrative (facilitating transfer but not energy requiring), moving solute down an electrochemical gradient (in this case, net movement from blood to brain), while others require energy to transport against a gradient, either derived directly from ATP (active transport, e.g. Na,K-ATPase) or from coupling to another molecule enabling use of a favourable concentration gradient (e.g. Na<sup>+</sup>-coupled, secondary active transport). Some transporters are located on both the lumen facing (luminal, apical) and the brain facing (abluminal, basal) membranes of the endothelium, while others are localised predominantly to one or other membrane, allowing the possibility of directional or vectorial transport. Thus the Na-dependent glutamate transporters (EAAT1-3) appear to be predominant on the abluminal membrane, facilitating glutamate removal from the brain [17]. Although the brain endothelial expression of many transporters is known, the apical/basal distribution is in many cases still unclear, which has complicated modelling of the kinetics of transport. Moreover, the cellular location of other relevant proteins such as enzymes (e.g. hexokinase in the case of glucose transport) may play an important role in vectorial solute flux [18].

4.4. Efflux transporters

As noted above, some of the uptake transporters of the brain endothelium may work bidirectionally, also subserving efflux. However, in addition, several families of transporters have been identified that are capable of transporting solutes out of the brain endothelial cells, often with consumption of ATP. These include the ABC (ATP binding cassette) family (P-glycoprotein (ABCB1), the Multidrug Resistance Related Proteins
(MRPs, ABCC1, 2, and 5) and the Breast Cancer Resistance Protein (BCRP, ABCG2)) [19]. Their roles in normal physiology are unclear, but they are able to restrict the CNS entry of a number of potentially harmful or toxic or lipophilic agents circulating in the blood, derived from the diet or metabolism, and may have other housekeeping functions. They have a particular significance for drug delivery, since they can effectively block or reduce entry of lipophilic drug molecules.

4.5. Transcytosis: receptor-mediated (RMT) and adsorptive-mediated (AMT)

Most standard membrane transporters are able to handle only small molecules (<1000 MW) because of the spatial and energetic requirements of the substrate-to-transporter docking sites and transfer mechanism; however, the broader specificity ABC family (e.g. P-glycoprotein) with less rigorous selectivity site(s) may not be so restrictive (e.g. transferring β-amyloid, MW~4500 [20]). Larger molecules such as polypeptides and proteins (e.g. transferrin, iron binding protein) can be transported across the BBB via vesicular routes. Two mechanisms have been identified [6,21]. One requires specific interaction with a membrane receptor followed by internalisation (endocytosis) and transfer across the cell (transcytosis)—receptor mediated transcytosis (RMT). The other is less specific, by which cationic molecules can bind to the surface negative charges of the endothelial glycocalyx and be internalised and transferred: adsorptive mediated transcytosis (AMT). This endocytotic traffic is less active in brain endothelium than non-brain endothelium, consistent with the low protein content of ISF and CSF, however, it may be critical for the transfer of small amounts of highly potent regulatory molecules such as TNFα [22]. These mechanisms offer promise for the delivery of agents too large to couple to membrane transporters.

4.6. The enzymatic barrier

The role of the liver in metabolising circulating compounds has been intensively investigated to elucidate the mechanisms contributing to the plasma pharmacokinetics of compounds. However, for CNS delivery, it is important to take into account further metabolism, particularly in the cells of the barrier layers, chiefly the brain endothelium and the choroid plexus. Both sites express a range of Phase I, II and III enzymes and, in the case of some (e.g. monoamine oxidase), the activity per gram tissue may approach that of the liver [23]. The influence of brain endothelial enzymes has to be considered on a case-by-case basis—thus although certain nucleoside analogues of value as neuroprotectants may be substrates for nucleoside transport at the BBB [24,25], their brain concentration will be strongly dependent on the their rate of metabolism by adenosine kinase and deaminase [26].

5. Induction of the BBB phenotype

Many of the features of the brain endothelium relevant to BBB molecular traffic are sufficiently distinct from those of peripheral endothelium (the default condition) to warrant inclusion in the term ‘BBB phenotype’. This involves the up-regulation of a number of mechanisms (e.g. tight junction proteins, transporters) absent from peripheral endothelium or expressed there at low levels [27]. The ability of astrocytic glia to induce many aspects of the BBB phenotype is well documented [11] and
recently it has become clear that other cells from the neurovascular unit (pericytes, macrophages) can also act as inducers [2]. Interestingly, the induction also involves down-regulation of some features (expression of MRP1, clotting factors), suggesting that a very particular combination of membrane proteins is optimal for BBB physiology. The detailed mechanisms by which the induction occurs are not clear, although it is known that components of the basal lamina (especially agrin) may be important in induction of both endothelial features and specialisations in the apposed astrocytic end feet [8]. It will be particularly interesting to discover whether the endothelium within neurovascular units in different parts of the brain can be subject to different inductive signals, resulting in regional differences in BBB properties. There is already some evidence for differences in BBB transport function, e.g. between brain and spinal cord [28] and between cerebellum and cerebral cortex [29].

6. Physiological regulation and modulation of the BBB

The BBB is not a fixed or static structure but dynamic, over evolutionary time, during development and in adult life [2]. This dynamic function is reflected in all aspects of the normal BBB phenotype described above. Thus co-culture with astrocytes changes the fatty acid (FA) composition of the endothelial membranes [30]; moreover, astrocytes were found to supply FA to the endothelial cells [31]. Regulation by signal transduction pathways downstream of endothelial receptors has been reported for several BBB transporters, including GLUT-1 and MCT-1, the monocarboxylate transporter [32,33], and changes in membrane fatty acids can also influence BBB transporter function [34]. Many of the receptors present on brain endothelial cells are able to increase the tight junctional permeability of the endothelium, by either Ca\(^{2+}\)-dependent or independent processes, and some are able to tighten the barrier, generally by elevation of intracellular cAMP [11]. This gives the endothelium a repertoire of possible regulatory mechanisms by which it can respond to changing requirements of the brain and in particular the neurons, possibly by differential responses to signals from different cell types of the neurovascular unit [9,10]. Much of the work so far has used cell cultured preparations, but it has been possible to observe similar phenomena in exposed pial surface vessels of the brain [35] and recent work on brain slices has demonstrated communication within the neurovascular unit [36].

7. Routes across the blood–brain barrier: drug delivery

From the physiology outlined above, it is clear that there are several potential routes for drug delivery to the brain (Fig. 1a).

Under normal conditions the tight junctions severely restrict penetration of polar (hydrophilic) molecules, but they may act as a route for leucocyte traffic; such cells may have the ability to unzip the junction locally as they transmigrate, with minimal leakage, or to adhere in the region of the junction then migrate through the cell [1,37]. However, given the low volume of leucocyte traffic across the BBB, these cells do not make a suitable drug delivery vehicle.

Many lipid-soluble drugs can diffuse through the endothelial cell membranes, although the nature of these membranes may place limits on permeation [13]. Nevertheless, drug
lipidisation and pro-drug strategies remain favoured modes of increasing drug delivery to the brain [7] and a number of in silico modelling approaches are able to give a reasonably good prediction of passive permeation via this route [38].

Carrier-mediated entry on uptake transporters has been exploited for delivery of a number of drugs, including L-DOPA and Gabapentin [7]; the L-system amino acid carrier is particularly suitable given its ability to accept a relatively large hydrophobic moiety on the drug. Many of the efflux transporters are located on the luminal membrane of the brain endothelium, tending to reduce entry of drug substrates for these transporters; the proportion of marketed drugs that are P-gp substrates is lower

Fig. 1. (a) Permeability and transport across the blood–brain barrier. TJ, tight junction. Modified from Begley [7]. (b) Some of the cell types present at the BBB capable of modulating brain endothelial permeability and function; the arrows indicate that modulatory influences may come from either the blood or the brain side.
for CNS drugs than for non-CNS drugs [39]. Hence there is great interest in the three strategies—reducing affinity for efflux transporters, blocking P-gp function, and bypassing P-gp by use of vehicles not detected by the transporters [7,40,41]. Of these, the second may be impracticable except in severe disease states, given the importance of P-gp in other sites such as the GI tract, liver and kidney. Major challenges are determining the SAR of BBB transporters allowing accurate predictive modelling, determining the relative expression levels and activities of transporters in the human BBB (extrapolation from animal studies may not always be justified), and modelling the pharmacokinetics in both blood and brain for any new compound of interest, ideally even before synthesis.

Considerable progress has been made in understanding vesicular mechanisms (RMT and AMT) as a route for large molecular delivery, with potential for clinical applications [21]. The main challenges will be in establishing safety, increasing drug ‘cargo’ and reducing cost.

Modulation of tight junction permeability may allow transient barrier opening (Fig. 1b); this occurs in some pathologies but may also be important physiologically. A low level activity of this kind, appearing as a stochastic process, with transient punctate sites of increased permeability ‘flickering’ over the microvascular wall could help to explain the observation that ~4% of tight junctions in immunocytochemical examination of normal brain show signs of opening [42]. It is necessary to distinguish between transient opening which may reflect physiological mechanisms and be well tolerated [43] and chronic opening which may lead to serious CNS pathologies [44,45]. A promising method for transient opening following controlled nerve stimulation [46] may prove of clinical value, particularly for delivery of complex molecules too large or polar to use other routes.

8. Role of BBB in ISF generation: effect on drug pharmacokinetics

The BBB is the likely site for generation of brain ISF, with fluid secretion driven by the Na,K-ATPase on the abluminal membrane [3]. Drug concentrations in brain will be influenced by the dynamics of this fluid production and flow; the effect will be most marked for polar molecules of low BBB permeability since their concentration in ISF will be reduced by the diluting effect of the new ISF secretion. The anatomical routes and dynamics of flow within the brain are also relevant for targeted therapies, e.g. to deliver growth factors or stem cells to sites of regeneration or repair [1,3].

9. Pathologies involving BBB dysfunction

There is a growing list of pathologies involving alterations of BBB function, which need to be taken into account in developing therapies. Any element of the BBB can be affected and some pathologies involve multiple deficits. Thus an open BBB can be found in some tumours [4] and acute phases of multiple sclerosis [47,48], with cellular infiltration and an inflammatory response, while in epilepsy both barrier opening (during seizures) and up-regulation and changed distribution of drug resistance efflux transporters have been reported [49,50]. For many pathologies, directing therapies to
protect or repair the endothelium may prove an effective way to reduce the severity of neurological symptoms or delay onset of neurodegeneration.

10. Conclusion

Better understanding of the physiology and pathology of the BBB, of potential routes for drug penetration at the BBB, and of ISF flow will improve the ability to apply ‘intelligent design’ to the generation of compounds for effective CNS targeting. The same principles can guide the design of drugs to avoid the brain, for compounds intended to act peripherally.

References


Discussion

De Boer

I’m very interested in how the evolution of those barriers went on. You say that those barriers started with glial barriers and then automatically ended up in endothelial barriers. Could you extrapolate that into the future when more sophisticated barriers could eventually develop?

Abbott

That’s an impossible question to answer; these things happen over millions of years. However, recent work by Magnus Bundgaard in Copenhagen in ancient fish groups adds to the evidence for an evolutionary transition from a glial to an endothelial barrier. In addition, the endothelial barrier must have evolved several times, suggesting it gave significant advantage [2]. In modern animals, elasmobranches including sharks still have a glial barrier, yet they survive and compete pretty well—that may be because they are often big fast carnivores who just eat the opposition, even if the opposition has an endothelial barrier. As for BBB evolution, it is very unlikely that in our lifetime we will see much change. Supposing it turns out that a particular BBB efflux transporter profile gives a significant evolutionary advantage because the animal or human with that profile is better able to efflux environmental toxins and damaging drugs from the brain, one might predict this transporter profile would become the predominant one as a result of natural selection. However there is problem with human evolution since many of these subtle differences may only give cumulative advantage over long periods (e.g. in old age), well beyond the peak reproductive age, and hence would have little selective advantage unless they also enhanced fertility or disease resistance. However it is useful to start thinking in evolutionary terms, particularly in relation to environmental pollution and the long-term consequences.

Kreuter

I have a question concerning the rapid change in permeability. I saw some very interesting pictures by Hartwig Wolburg who was closely working together with Britta Engelhardt, and he says the cells that penetrate from the blood into the brain never go through the tight junctions, they always get sort of endocytosed and transcytosed [37]. And, therefore, I ask you, maybe these rapid changes in permeability (Easton et al. 1997, J. Physiol. 503:613–23) are an event occurring when a cell is just penetrating through the endothelial cell. This cell penetration seems to occur quite rapidly. So this also could be a mechanism where the barrier function is compromised while the cell penetrates through the blood–brain barrier?
Abbott

This is an important point—both routes are clearly important, but we are still uncertain which molecules or cells use which route, and whether this varies depending on local conditions. Members of the JAM family of 'junction associated molecules' are implicated in the attachment of leucocytes to the endothelium, before migration, and JAM1 is clearly expressed in the mouth of the tight junction (Vorbrodt and Dobrogowska 2003, Brain Res Rev. 42:221–2). It may be that the tight junction is a convenient landmark or attachment site facilitating the docking and adhesion of cells before they transmigrate. In terms of evidence for opening of the tight junctions, this can be observed even in the absence of adherent leucocytes or diapedesis, so it certainly can occur. The advantage of cells migrating across the endothelium via the cells rather than the junctions is that this could be a relatively 'silent' passage, easier to seal up the breach as the cell migrates and hence not disturbing the homeostatic function of the barrier. Nevertheless we now have abundant evidence for modulation of the cytoskeletal proteins controlling the tight junctions, in response to a number of signals that can be produced from both the blood side and the brain side, e.g. from neurons. We now need to establish what controls access to these two routes across the endothelium.

Gabathuler

I have a question about species differences, basically from an industry perspective because as far as possible one tries to get models that mimic the human situation. However, a mixture of models is often used, e.g. bovine brain capillary endothelial cell monolayers to predict BBB drug permeability, followed by a rodent animal model for more detailed study of drug PK and metabolism, and finally one tries to predict drug behaviour in humans. For receptor-mediated transcytosis of potential novel drugs, what would be the best model to use, or must one use a variety of models?

Abbott

Like many of the participants at this meeting we have been trying to optimise in vitro models of the BBB, to the point where they can be good predictors for the human BBB. For example we have used the human ECV304 cell line with a mixed endothelial—epithelial phenotype, to test whether it would perform better as predictor than bovine or rodent models. It does a pretty good job but appears not to be as robust as some other cell lines or primary brain endothelial models. There has been some progress in making human brain endothelial cell monolayers tight enough for drug permeation studies, but there are still large problems with yield and reproducibility. A major problem is that we have too little information on the human BBB to act as a reference or gold standard—we don’t know what we are aiming for. We can’t easily or ethically get a biopsy of the BBB from a particular patient to establish their brain endothelial transporter profile, which might be necessary for designing a good ‘surrogate’ BBB model in vitro. I think Dr Tsuji is going to mention human and rat differences—is this correct?

Tsuji

Yes, I am planning to talk on that issue, but here, I’d like to bring up that Dr. Partridge mentioned about P-glycoprotein not expressed in mammalian cells, such as monkeys. The higher animals do not express it at the blood–brain barrier, they express it at the astrocytes. So the species difference may occur. What do you think about this?
Abbott

It is true that Bill Pardridge and Pam Golden published some studies which suggested that BBB P-gp was mainly located in the endfeet of perivascular astrocytes not in the endothelium itself (Golden and Pardridge 1999, Brain Res. 819:143–6). However, this may have been partly artefactual, or reflected abnormal up-regulation of astrocytic P-gp, as seen in some pathologies. Certainly recent confocal study does find brain endothelial P-gp localisation in rhesus monkey brain (Schlachetzki and Pardridge 2003, NeuroReport 14:2041–6), so this debate is now largely resolved. The recent identification of other efflux transporters at the BBB including BCRP (ABCG2) complicates matters—a full understanding will require information on the relative BBB expression levels in different species, on any differences in substrate specificity, and on changes resulting from pathology. We need to know which transporters exert most effect, and which are critical for drug delivery.

Du Souich

One comment and two questions. I think there are ways to assess the expression of these transporters by looking at peripheral sites, for instance the GI tract, which will allow us to phenotype and genotype patients. The first question is, in your slides showing the expression of MDR1 in epilepsy, were these subjects under treatment?

Abbott

This material from Damir Janigro’s group (Marroni et al. 2003a, Current Drug Targets 4:297–304) was from patients undergoing surgical resection for temporal lobe epilepsy. In most micrographs there is a small zone of normal-appearing brain outside the pathological area that can be used as control, to assess the level of normal transporter expression, and any up-regulation.

Du Souich

What does age do to the blood–brain barrier?

Abbott

It depends whether we are talking about normal ageing, or ageing with related pathology such as Alzheimer’s. In normal ageing, for example in a 90-year old with good mental faculties and intact sensory and motor function, the BBB is likely to be pretty healthy because the neurons are clearly working well. Generally standard neuroimaging techniques such as MRI and CT scanning do not pick up overt leaks in aged individuals, but small increases in permeability, whether focal or more diffuse would be hard to detect, and some changes in tight junctional proteins have been reported in experimental models (Mooradian et al. 2003, Mech. Ageing and Devel. 124: 143–6). It is much more difficult to assess transporter function non-invasively. In Alzheimer’s there are reported changes in both BBB permeability and the GLUT-1 glucose transporter (Kalaria 2002, Cerebrovascular Diseases 13, Suppl 2:48–52), but in many neuropathologies of old age it is not clear whether BBB disorder is cause or effect. What is known is that experimentally induced increase in BBB permeability in a rat model can lead to epileptic activity [45]—an indicator that change in BBB function can have serious effects on neurons. We need more sensitive assays to detect slowly developing BBB dysfunction in animals and humans.

Gabathuler

Are there changes in BBB permeability between the awake and sleeping states?
Abbott

As far as I know we don’t have this information. The problem is that most human BBB imaging methods are relatively invasive and have not been applied to this kind of study. In any case, differences are likely to be small, and maybe focal, so difficult to detect. There is some evidence for differences in transporter activity according to a diurnal rhythm, e.g. for TNF–leptin [28], but it is not clear whether this is related to changes in blood flow, or hormones, or something else. There is much more to learn.

Stanimirovic

In addition to the presented list of compounds that might open the blood–brain barrier through receptor mediated mechanisms, I wonder if you could comment on modulators of cell cycle and blood–brain barrier opening. Compounds or growth factors that stimulate cell cycle in endothelial cells, for example VEGF, are also known to open the blood–brain barrier.

Abbott

I haven’t included in the list agents that act predominantly on cell division and the cell cycle, because in the normal adult brain endothelium there is very low cell turnover, with a lifetime of ~120 days or longer, which means that at any one time a very small proportion of the cells will be dividing. It is indeed possible that the rounding up of cells and separation from neighbours during the process of cell division will temporarily create small holes across the BBB, which may contribute to the figure of ~4% open or incomplete tight junctions [42] (Kirk et al. 2003, J. Pathol. 201: 319–327). It is certainly possible to show that VEGF$_A$ increases permeability of in vitro BBB models, but I would class it as a growth factor involved in proliferation and angiogenesis (new vessel growth) during development and repair (Krum and Khaibullina 2003, Exp. Neurol. 181:241–57) rather than a physiological modulator. There is evidence for down-regulation of VEGF$_A$ receptors with differentiation and maturation of the brain endothelium, while VEGF$_B$ may be required for BBB maintenance (Nag et al. 2002, J Neuropath Exp Neurol 61: 778–88).

Stanimirovic

In many pathologies, such as brain tumours and ischemia, several molecular events can start cell proliferation, and we believe that a coordination of signals that induce cell cycle and signals that induce differentiation of endothelium and tight junctions is necessary in order to achieve remodelling of vessels. It appears that the stimulation of cell cycle in brain endothelium is always accompanied by the blood–brain barrier opening.

Abbott

You would then predict that once the remodelling is complete, these signals would be switched off, again in a regulated way, to allow the differentiation phase to take over. Astrocytes appear to play a key role in producing or regulating the differentiating signals that lead to establishment of the barrier [11].

Whittle

I was very interested in your comment about chronic barrier opening leading to epilepsy in rodents, because in humans with malignant brain tumours, which have the most florid barrier opening, the incidence of epilepsy isn’t that high, whereas with lower grade brain tumours, which have less barrier opening, you get much more epilepsy.
That is interesting. The work from Alon Friedman's group is quite recent [45], and the only measure of epilepsy was abnormal electrical activity. They showed increased neuronal excitability and epileptiform activity in brain slices from barrier-opened compared with control rats, possibly caused by exposure to serum albumin. However, the detailed mechanisms need to be examined. The human tumour data is intriguing—is it possible that high grade tumours may cause activation of some of the protective mechanisms present, whether a 'glial scar' isolating the tumour from the surrounding brain, or release of agents from the tumour suppressing neuronal excitability. You are right, it is unlikely there will be a simple correlation between barrier opening and degree of epilepsy—bearing in mind there are a large number of different types of epilepsy syndrome and over 30 different types of seizure are recognised. It would be important to study groups of patients with the same syndrome when examining association with BBB opening.

Dr. Pardridge mentions that more than 98% of drugs do not cross the BBB. For me this is surprising. What do you think about this?

Dr. Pardridge is a very prolific writer, and he often quotes this figure when reviewing drug delivery to the brain. I think it dates from estimates in the mid 1990s when combinatorial chemistry was beginning to be able to generate a large number of new compounds, yet companies were not as aware as they are now of issues such as delivery across the BBB, so many potential drugs were unsuitable for this purpose. I think understanding has improved since then, with more focussed programmes on CNS drug delivery.

It is difficult to answer this question since it depends, of course, on the stage of development. The fraction of 98% could correspond to a very early discovery phase, however this number is overestimated when applied to the preclinical development phase.

Exactly. I think the figure has been useful in prompting pharmaceutical companies to take seriously the issue of BBB properties early in the drug discovery and design process; the figure helped to shock companies into realising there could be a problem. I think companies are now doing much more engineering of molecules for CNS targeting early in the discovery phase.

Are there any kinds of congenital disorders of blood–brain barrier formation? You mentioned the importance of the astrocytic end foot and other trophic factors, perhaps from neurons, and quite clearly when the blood–brain barrier develops in infancy or in the embryo, there must be factors inducing it. Do you know of any dysfunction in this process that has led to some evidence of pathology, even in dead foetuses?

Scientists working in the blood–brain barrier field sometimes complain that we may be seen as a rather small community because there is no obvious 'blood–brain barrier disease', unlike a condition such as diabetes where there is a very clear anomaly. But it is increasingly
obvious that many neuropathologies do have some blood–brain barrier dysfunction element. In the epilepsy field, it is becoming clear that many of the observed dysplasias are congenital, and can appear in infants or aborted foetuses. If there is an astrocytic abnormality so the blood–brain barrier doesn’t form properly, these sites may become epileptic foci later, with up-and down-regulation of certain BBB features as described in the literature (Marroni et al. 2003a, Curr. Drug Targets 4:297–304), but I think this field has not yet been very well explored.

Whittle
Yes, I think you might be spot on, because there’s a disorder called dysembryoplastic neuroepithelial tumour, which is thought to be congenital. It’s really a haematomatous malformation of cortex and glia, and on MRI, is quite often enhanced which suggests there is a gadolinium leakage across the blood–brain barrier. When one looks for proliferative markers, one does not detect any proliferative activity at all, and they are very epileptogenic. That’s how they present.

Abbott
That’s very interesting. One thing that has come out of the work from the Janigro group is the similarity in many respects between the pattern of gene expression in tissue from epilepsy brain and from brain tumours (Marroni et al. 2003b, Neurosci. 121:605–17). A possible view is that many epilepsies developing later in life are actually congenital, derived from a low-grade tumour that only causes problems later. There may be low proliferation but they have the same sort of abnormalities at the cellular level. These kinds of comparison are very interesting and may lead to the conclusion that there are some underlying similarities between disorders that present in rather different ways.