The Blood Brain Barrier
Learning objectives

• Basic understanding of BBB structure and role structure plays in function
• Transport mechanisms
• Impact of BBB disruption (links in with future lectures)
• Appreciation of the obstacle BBB poses for drug delivery
• **1885:**
  – Paul Ehrlich notes that trypan blue injected *i.v* stain all organs EXCEPT the brain and spinal cord
  – Attributes this to an inability of nervous tissue to take up the dye

• **1900:**
  – M. Lewandowsky coins the term “*bluthirnschranke*” (blood brain cabinet) while studying the penetration of potassium ferrocyanide
• 1913:
  – Edwin Goldmann (Ehrlich’s student) injects water soluble dyes directly into the CNS

Figure 1. The Blood-Brain Barrier, or BBB, to Trypan Blue and Its Diffusion from the Cerebrospinal Fluid, or CSF, into the Brain
(A) Goldmann’s first experiment. Trypan blue (dye) was injected into the blood. Brain and CSF were analyzed.
(B) Goldmann’s second experiment. Dye was injected into the CSF. Brain and blood were analyzed.
(C) Conclusions from the two experiments.
What is the blood brain barrier (BBB)?

• The brain is a privileged site, sheltered from the systemic circulation by the blood-brain barrier (BBB)
• Highly specialised brain endothelial structure of the fully differentiated neurovascular system
Why study the BBB?

- Problem of drug delivery
- Role of BBB in the pathophysiology of CNS diseases
  - Brain not “immune-privileged” (relative)
  - Understanding the anatomy and cell biology of the neurovascular unit in health and disease is critical for advancement of therapeutic development
Neurovascular System

- Normal neuronal-vascular relationship is critical for normal brain functioning
- Estimated that every neuron has a capillary
- Human brain: total length = 650 km
- Capillary surface area available for molecular transport = 20 m²
- Length of brain capillaries is reduced in neurodegenerative disorders (e.g. AD)
- Vascular reductions an diminish transport of energy substrates and nutrients across the BBB and reduce the clearance of potential neurotoxins from the brain
THE PRIMARY ROLE OF THE BBB

It separates components of the circulating blood from neurons and so maintains the chemical composition of the neuronal microenvironment.

A stable microenvironment is required for proper functioning of neuronal circuits, synaptic transmission, synaptic remodeling, angiogenesis, neurogenesis.

It protects the brain from pathogens and endows relative immune privilege.
BBB – dual function

- **Barrier** function and a **Carrier** function

- **Barrier function** – 4 main
  - **Paracellular barrier**
    - Formed by endothelial junctions restricts the free movement of H2O sol compounds
  - **Transcellular barrier**
    - Made possible by low level endocytosis & trancytosis -> inhibits transport of substances to the cytoplasm
  - **Enzymatic barrier**
    - Complex set of enzymes, including acetylcholinesterase, alkaline phosphatase, gamma-glutamyl transpeptidase, monoamine oxidases & other drug metabolizing enzymes capable of degrading different compounds
  - **Cerebral Endothelium**
    - Expresses a large number of efflux transporters (ABC, ATP-binding cassette transporters like ABCB1 (p-glysoprotein) etc
Carrier function

• NB function – responsible for the transport of nutrients to the brain and the removal of metabolites
  – Small lipid soluble molecules and blood gases like O2 and CO2 diffuse passively through the BBB while essential nutrients like glucose and amino acids require specific transport proteins in order to reach the brain
Cellular components of the BBB

- Principal components are:
  - Endothelial cells
  - Astrocytes
  - Pericytes
- Other cellular components like neurons and microglia also play significant role (immune function)
Endothelial cells

- From point of view of permeability → most NB cell type = Brain Endothelial cells
  - Form a continuous sheet covering the surface of capillaries
  - Inter-connected by TIGHT JUNCTIONS (x50-100 tighter than periphery) – form belt like structure at the apical region of the cells
Different types of endothelia

- **Fenestrated Endothelia**
  - e.g. Kidney
  - thick CNS membrane
  - presence of circular pores of fenestrae that penetrate the endothelium
  - => allow passage of small macromolecules through endothelium

- **Sinusoidal Endothelia**
  - e.g. Liver
  - do not form a CNS lining between the lumen & surrounding tissues
  - Gaps between adjacent endothelial cells
  - discs/absent basement membrane
  - => Poses no barrier to blood constituents

- **Continuous Endothelia**
  - e.g. Brain
  - CNS basement membrane
  - CNS endothelial membrane "no fenestrae"
  - TJ
  - => Restricts passage of most substances across endothelium
Anatomical site of the blood-brain barrier (BBB)

A. Brain capillary

Schematic drawing of the ultra structural aspects of one brain capillary (a) and one general capillary (b).

The endothelial cells (EC) of cerebral capillaries are connected with tight junctions and normally do not contain microvesicles for vesicular transport as compared to the non cerebral capillary. The endothelial cells of the cerebral capillaries are also covered with a thick layer of basement membrane (BM) compared to the general capillary.
Brain endothelial cells (ECs) differ significantly from non-brain ECs

• (i) the absence of fenestration correlating with the presence of intercellular tight junctions (TJs),
• (ii) the low level of non-specific transcytosis (pinocytosis) and paracellular diffusion of hydrophilic compounds,
• (iii) a high number of mitochondria, associated with a strong metabolic activity
• (iv) the polarized expression of membrane receptors and transporters which are responsible for the active transport of blood–borne nutrients to the brain or the efflux of potentially toxic compounds from the cerebral to the vascular compartment
Astrocytes

- NB components of BBB – capable of inducing BBB properties in endothelial cells
- Endfeet of astrocytes cover significant part of endothelial surface
- Astrocytes NB source of regulatory factors – TGF-β, GDNF, IL-6
- Influence BBB
Astrocyte: Endothelium co-culture

- TEER
- = transendothelial electrical resistance
- Measure of TJ “tightness”

Closing the gap between the in-vivo and in-vitro blood–brain barrier tightness

Katayun Cohen-Kashi Malina, Itzik Cooper, Vivian I. Teichberg

BRAIN RESEARCH 1284 (2009) 1-21
Pericytes

- Endothelial cells are sitting on the basal membrane – engulfed in basal membrane are the pericytes
  - Derived from the Greek word “kytos” (hollow vessel), pericytes surround small vessels.
  - Cover 22-32% of endothelium
  - Play NB role in regulation of endothelium proliferation, angiogenesis and inflammatory processes
  - Regulate BBB-specific gene expression patterns in endothelial cells
  - Unduce polarisation of astrocyte endfeet surrounding CNS blood vessels
  - In the absence of pericytes - an abnormal vasculogenesis, endothelial hyperplasia and INCREASED permeability in the brain
Other cellular components

• **Neurons** not directly involved structurally in formation of BBB, cerebral capillaries are innervated by different noradrenergic, serotonergic, cholinergic or GABA-ergic neurons

• Neurons regulate NB aspects of BBB function – can induce expression of BBB related cytokines in cultured cerebral endothelial cells

• **Microglia** found in perivascular space playing NB immunological role
  - Contribution to BBB properties not well characterised
Tight Junctions (TJs)

- formed by an intricate complex of transmembrane proteins (junctional adhesion molecule-1, occludin, and claudins) with cytoplasmic accessory proteins (zonula occludens-1 and -2, cingulin, AF-6, and 7H6).
- They are linked to the actin cytoskeleton, thereby forming the most intimate cell to cell connection.
- The TJ are further strengthened and maintained by the interaction or communication of astrocytes and pericytes with brain endothelia cells.
Tight Junction

Plasma Membrane

Cytoplasm

Apical

Basolateral

Occludin

Claudins

Cingulin

α-actinin

Cadherins

Catenins

Vinculin
Modulation of BBB function

• “BARRIER” suggests relatively fixed structure
  – BBB phenotype subject to change/modulation
  – Examples:
  – Opening of BBB’s tight junctions can occur in inflammation, contributing to brain oedema
  – Upreg of GLUT1 transporter exp observed in starvation & hypoxia
  – Some of the inflammatory mediators that increase capillary permeability in periphery (histamine, bradykinin) also act on brain endothelium
Physiological Adv to TJ modulation

• Opening triggered by histamine released from nerve terminals to allow passage of growth factors & antibodies into brain from plasma

• Tightening of barrier NB in conditions of stress or hypoxia
  – Conditions in which intracellular cAMP conc increased can lead to increased TEER and upreg of Pgp activity
Box 1 | **Agents modifying brain endothelial function and BBB tightness**

A number of chemical agents circulating in the plasma or secreted from cells associated with the blood–brain barrier (BBB) are capable of increasing brain endothelial permeability and impairing its transport and metabolic functions\(^{56,68}\). Other agents have the opposite effect, improving tightness and BBB function.

**Agents that impair BBB function:**

- Bradykinin, histamine, serotonin, glutamate.
- Purine nucleotides: ATP, ADP, AMP.
- Adenosine, platelet-activating factor.
- Phospholipase A\(_2\), arachidonic acid, prostaglandins, leukotrienes.
- Interleukins: IL-1\(\alpha\), IL-1\(\beta\), IL-6.
- Tumour necrosis factor-\(\alpha\) (TNF\(\alpha\)), macrophage-inhibitory proteins MIP1 and MIP2.
- Complement-derived polypeptide C3a-desArg.
- Free radicals, nitric oxide.

**Agents that cause barrier tightening and improved function:**

- Steroids, elevated intracellular cyclic AMP, adrenomedullin and noradrenergic agents.
Transport Mechanisms – a closer look
**Paracellular (aqueous) diffusion**

- Diffusion of substances between the cells is termed as paracellular diffusion.
- It is non-saturable and non-competitive. In brain, however, it does not occur to any great extent at the BBB, due to the “tight junctions”.
- Only small water-soluble molecules can diffuse through the BBB by apparently passing through the tight junctions.
Transcellular (lipophilic) diffusion

- Diffusion of substances across the cells is termed as transcellular diffusion.

- Similar to paracellular diffusion, it is also non-saturable and non-competitive. In the case of transcellular diffusion, the general rule is the **higher the lipophilicity of a substance along with a molecular weight less than 450, the greater the diffusion into the brain**

- If two substances, identical on all other fronts, vary in molecular weight, the smaller substance will penetrate more rapidly; consequently small inorganic molecules (i.e. O₂, CO₂, NO, and H₂O) are highly permeable across the endothelial cells by dissolving in their lipid plasma membrane.

- Additionally, **hydrogen bonding property** is also a major determining factor. Since hydrogen bonding is primarily associated with oxygen and nitrogen moieties in a molecule, then, if the sum of the nitrogen and oxygen atoms in the molecule is five or less, then the molecule has a high probability of entering the CNS.
Transport proteins /carrier-mediated transport

- Binding of a solute such as glucose or amino acids to a protein transporter on one side of the membrane that triggers a conformational change in the protein, resulting in the transport of the substance to the other side of the membrane, from high to low concentration.
- If compounds need to be moved against a concentration gradient, ATP may provide the energy to facilitate the process.
- **Efflux pumps** or transporters are responsible for extruding drugs from the brain and this mechanism is a major obstacle for the accumulation of a wide range of biologically active molecules in the brain, with the ATP binding cassette (ABC) transporter P-gp and multidrug resistant protein (MRP) being the principle efflux mechanism of these agents.
- Inhibition of P-gp in pre-clinical studies has enhanced the penetration of paclitaxel* into the brain, indicating the feasibility of achieving improved drug delivery to the brain by suppression of P-gp.

*mitotic inhibitor used in cancer chemotherapy
Adsorptive-mediated transcytosis (AMT)

- The stage of transcytosis at the BBB starts with uptake either through clathrin-coated pits or caveolae.
- Transcytosis of molecules at the BBB is an **energy requiring/ATP-dependent transport process**, both for the endocytosis of the transported molecule at the luminal side of the EC and for its transport across the EC as well as for its exocytosis at the basolateral side.
- The **density of mitochondria** in cerebral EC is roughly **five times greater** than in peripheral endothelia, increasing the energy potential of the BBB as well. This enhanced cerebral capillary work capacity may be related to energy-dependent transcapillary vesicular transport.
- AMT may not involve specific plasma membrane receptors and that endocytosis is initiated through charge–charge interaction between polycationic substances and negative charges on the endothelial surface.
The transport of peptides and proteins across cellular barriers has been documented in a number of systems like insulin, insulin-like growth factors (IGF-I, IGF-II), angiotensin II, atrial and brain natriuretic peptide (ANP, BNP), IL-1 and transferrin.

However, receptor-mediated endocytosis across the BBB in vivo has been shown for few peptides and proteins like insulin, transferrin, certain cytokines and leptin while angiotensin II and ANP may exert their effects by binding on the luminal cytoplasmic membrane of brain microvessel endothelia, and may even be involved in the regulation of BBB permeability for other substances.
BBB AND DISEASE
Pathological states involving BBB breakdown or disorder

Stroke
- Astrocytes secrete transforming growth factor-β (TGFβ), which downregulates brain capillary endothelial expression of fibrinolytic enzyme tissue plasminogen activator (tPA) and anticoagulant thrombomodulin (TM) 150.
- Proteolysis of the vascular basement membrane/matrix 151.
- Induction of aquaporin 4 (AQP4) mRNA and protein at BBB disruption 152.
- Decrease in BBB permeability after treatment with arginine vasopressin V1 receptor antagonist in a stroke model 153.

Trauma
- Bradykinin, a mediator of inflammation, is produced and stimulates production and release of interleukin-6 (IL-6) from astrocytes, which leads to opening of the BBB 180.

Infectious or inflammatory processes
- Examples include bacterial infections, meningitis, encephalitis and sepsis.
- The bacterial protein lipopolysaccharide affects the permeability of BBB tight junctions. This is mediated by the production of free radicals, IL-6 and IL-1β 154.
- Interferon-β prevents BBB disruption 155.

Multiple sclerosis
- Breakdown of the BBB 187.
- Downregulation of laminin in the basement membrane 156.
- Selective loss of claudin 1/3 in experimental autoimmune encephalomyelitis 94.

HIV
- BBB tight junction disruption 157, 158.

Alzheimer’s disease
- Increased glucose transport, upregulation of glucose transporter GLUT1, altered agrin levels, upregulation of AQP4 expression 95, 159.
- Accumulation of amyloid-β, a key neuropathological feature of Alzheimer’s disease, by decreased levels of P-glycoprotein transporter expression 160.
- Altered cellular relations at the BBB, and changes in the basolateral and amyloid-β clearance 100.

Parkinson’s disease
- Dysfunction of the BBB by reduced efficacy of P-glycoprotein 101.

Epilepsy
- Transient BBB opening in epileptogenic foci, and upregulated expression of P-glycoprotein and other drug efflux transporters in astrocytes and endothelium 94, 99.

Brain tumours
- Breakdown of the BBB 163, 162.
- Downregulation of tight junction protein claudin 1/3; redistribution of astrocyte AQP4 and Kir4.1 (inwardly rectifying K⁺ channel) 159, 158.

Pain
- Inflammatory pain alters BBB tight junction protein expression and BBB permeability 158.

NB review to link with next week’s lectures
Getting drugs across the BBB

- Intact BBB major obstacle
- Approx. 98% of small molecule drugs and all large nanotherapeutics excluded from the brain
BBB and therapy

- Diseases of the central nervous system constitute **38.35% of the global economic health burden**
- Psychiatric drugs do have one advantage over other CNS medications.
  - While most medications treating mental illnesses such as schizophrenia and depression are small-molecule, lipid soluble compounds capable of crossing the blood-brain barrier, other CNS treatments currently cannot do this.
  - Genes responsible for many of these disorders, including Alzheimer’s disease, Huntington’s disease, and Parkinson’s disease known, there is currently no way large enzymes can be delivered to the brain using gene therapy. In the case of Parkinson’s disease, L-Dopa, used to alleviate symptoms, can enter the brain, but provides only temporary relief.
Potential Solutions to the Blood-Brain Barrier Problem

• **‘trans-cranial’ drug delivery**, involves the medication being injected or inserted into the brain itself. Problems with this have arisen, however, because diffusion rates from the site of delivery are not rapid enough.

• **‘blood-brain barrier disruption’**, where solutes such as mannitol are used to shrink the brain’s endothelial cells, allowing various molecules to pass into the cerebral tissue. Trials to date, however, have revealed serious side effects associated with this treatment.

• **‘Trans-nasal’ drug delivery** to the brain via the nasal cavity has also been considered, but it has been found that this only works for certain small molecules and in limited quantities.

• Use of molecules such as **monoclonal antibodies** to act as molecular ‘Trojan horses’, may help to carry genes and large proteins across the blood-brain barrier.
  
  — Limited success with this technique has occurred in trials on rats with experimental Parkinson’s disease. In these trials, plasmids containing dopamine-producing genes were first encapsulated in liposomes and then attached to monoclonal antibodies, which transported the genes into the brain.

• Similar ‘Trojan horse’ molecules are also being tested with **nanocapsules** containing glial growth factors or neurotransmitters. If successful, these treatments may help to repair damaged nerve cells in the brain and spinal cord.
Conclusions

• BBB formed by brain endothelium under inductive influence of adjacent cell types
• Damage to BBB involved in many CNS pathologies either directly or via other cell types
• Major obstacle for drug development
Reading List

**The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders**

Berislav V. Zlokovic

**Astrocyte–endothelial interactions at the blood–brain barrier**

N. Joan Abbott *, Lars Rönnbäck * and Elisabeth Hansson *

**ENGAGING NEUROSCIENCE TO ADVANCE TRANSLATIONAL RESEARCH IN BRAIN BARRIER BIOLOGY**


**Inflammatary cell trafficking across the blood–brain barrier:**

chemokine regulation and in vitro models

© 2012 John Wiley & Sons A/S

Immunological Reviews 248/2012