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The Blood-Brain Interface

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Summary

The properties of the blood-brain barrier are those of the capillary endothelium in brain. This endothelium contrasts with that elsewhere in being sealed with tight junctions, having a high electrical resistance and low permeability to polar solutes. It is exceptional in having a paucity of pits and vesicles, a specialised enzyme content and a high density of mitochondria. Functionally, a range of transport mechanisms allow rapid movement of certain specific metabolic substrates. Ion pumps are concerned with secretion of brain interstitial fluid and regulation of its ionic concentration. The retinal capillaries are largely identical to those of the brain, but entry of solutes into retina is also determined by the properties of the pigment epithelium, functionally separating the retina from the highly vascular choroid. A clear difference lies in the greater resistance of cerebral microvessels to diabetic damage. The mechanism of this difference is unclear, but may relate to a better control of the brain interstitial fluid at a lower glucose concentration than is possible in the retinal interstitial fluid.

Exchange of solutes between blood and brain is largely dependent on the properties of the capillary endothelium which separates blood plasma and cerebral interstitial fluid. In contrast to that of capillaries elsewhere, this endothelium (the blood-brain barrier) is exceedingly 'tight' to non-lipid soluble substances and has many of the characteristics of a tight epithelium. Where brain tissue is close to ventricular or subarachnoid cerebrospinal fluid (CSF), solutes may exchange in either direction between brain and this fluid. Since CSF is separated from blood by the relatively tight cellular barriers of the choroid plexus epithelium and of the arachnoid matter, transport into and out of the whole system is limited as in the retina, eye fluids and lens. Substances which do not significantly cross the brain capillary wall, including colloid and particulate material, may slowly leave the system with the bulk drainage of CSF. Where brain tissue is not adjacent to CSF, exchange with blood is dominated by the route across the cerebral endothelium. This paper will concentrate on the properties of this transport. The topic has been extensively reviewed.[1,2,3,4,5]

Ultrastructure of brain endothelium

The morphological basis of the blood-brain barrier is the blocking of diffusion through the inter-endothelial spaces by occluding tight junctions. These junctions appear to be continuous[7] and not to be interrupted by gap junctions as in the endothelium of the carotic artery and of the aorta.[8] Additionally, there is a relative paucity of pits and vesicles in the brain endothelium. Frog pial capillaries have typical blood-brain barrier (BBB) properties. When these have been cryofixed rather than

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aldehyde-fixed, the pits and vesicles are even fewer.[7]

Besides, the above ultrastructural differences between brain endothelium and that of capillaries elsewhere, there are biochemical contrasts. Enzymes which are present in high concentration in cerebral endothelium include γ-glutamyl transpeptidase, alkaline phosphatase, Na-K-ATPase, Ca-ATPase, butyrylcholinesterase, aromatic-L-amino acid decarboxylase and monoamine oxidase. Overall this picture is more characteristic of an actively transporting epithelium than of a typical endothelium. The presence of the Na-K-ATPase and of the Ca-ATPase are at the basis of active transport across the blood-brain barrier. The former is mainly localised in the abluminal membrane and is probably involved in both regulation of the potassium concentration in brain interstitial fluid and in the primary slow secretion of this fluid. The Ca-ATPase is likely to play a role in calcium regulation in interstitial fluid and has been shown to mitigate against lead uptake from blood into brain.[9] The energy needs of such pumps are supported by numerous mitochondria. These are 5-6 times more numerous per capillary cross-section in brain than in skeletal muscle.[10]

Another characteristic feature of brain capillaries is their investment by the processes of astrocytes. These form a continuous sheath around all cerebral capillaries and are only separated from the endothelium by the perivascular basement lamina. Despite the 'continuity' of the sheath, there are 20 nm wide gaps between adjacent processes across which horse radish peroxidase and other solutes including proteins can readily diffuse. Thus the glial investment does not significantly contribute to the physical barrier. Recent experimental approaches, involving transplantation of tissues and ingrowth of blood vessels, indicate that the particular structural and functional properties of cerebral capillaries are determined by the surrounding central nervous tissue.[11]

The astrocytic end-feet are obvious candidates for mediating such an influence. Janzer and Raff[12] have transplanted isolated astrocytes into the anterior chamber of the rat eye. Some formed plaques growing on the walls of the chamber. Ingrowing capillaries acquired the barrier characteristics of brain capillaries. Besides inducing and maintaining the structure and function of brain capillaries, astrocytes may provide a route whereby the activity of neurones may influence endothelial contractility of permeability or both. They also appear to be involved in regulation of the potassium concentration in interstitial fluid adjacent to neurones.

Permeability of Brain Capillaries

A good index of the density of water-filled channels and hence of a route for transport of polar solutes across the BBB is its electrical conductance. This was measured again in the accessible frog pial capillaries.[13] The high resistance of the cellular membrane of 1,900 ohm.cm^2 was comparable to that of a tight epithelium such as amphibian skin or bladder or indeed to that of the cell membrane itself. Subsequent studies[14] have suggested that there may be a small number of neutral or weakly charged channels which are unable to discriminate between Na^+, K^+, and Cl^-. However, the very close correlation between permeability of a compound at the BBB and its lipid solubility,[3] indicates that the few water-filled channels across the barrier, if present, have little functional influence.

Hence, the permeability properties of the cerebral capillaries are largely those of the two plasma membranes, luminal and abluminal, of the endothelial cells themselves. Movement of a solute from blood to brain involves it crossing these two membranes in series. The permeability to polar non-electrolytes, such as mannitol or sucrose, is very low and that to the larger inulin insignificant. A number of metabolic substrates cross the plasma membranes of the cerebral endothelium via specific transport systems. Transport by each of these mechanisms is saturable, subjective to competitive inhibition and stereospecific. Table I contains a list of the major specific transport mechanisms and their kinetic constants. The systems for glucose, monocarboxylic acids and neutral amino acids are of particular importance and clinical relevance. Glucose transport is by facilitated diffusion rather than by an energy-dependent process. The relative affinities of the trans-

Table I *Various transport systems blood-brain barrier for physiologically important non-electrolytes*

Class of compounds and representative	*Maximum transport capacity (V_{max}) (umoles/g per min)*	*Apparent Michaelis Constant (K_m) (mM)*
Monosaccharides, D-glucose	2–4	7–11
Monocarboxylic acids, L-lactate	90	2
Neutral amino acids L-leucine	30–60	0.025–0.1
Basic amino acids L-arginine	8	0.9
Dicarboxylic amino acids L-glutamate	Low	—
Amine, choline	11	0.34
Nucleosides, adenosine	0.75	0.025
Purines, adenine	0.05	0.01

From various sources in the literature and recorded in Reference 6.

porter for different monosaccharides and for non-competitive inhibitors are very similar to those of the equilibrating system in the human red blood cell membrane and in the guinea-pig placenta. The properties of glucose transport are thus quite unlike those of the sodium-dependent uphill transport occurring in small intestine and in the renal tubule. Photo-affinity labelling with cytocholasin B has been used to separate the glucose transporter from isolated cerebral capillaries.[15,16] It appears to be an integral membrane with a molecular weight of 53,000. It cross-reacts with antiserum raised against the erythrocyte glucose transporter.

The activities of the three major transporters mentioned above may all be modulated in relation to the current metabolic conditions. The glucose transporter is substantially down regulated during streptozotocin-induced hyperglycaemia in the rat.[17] Conversely, unidirectional glucose influx into brain is maintained when plasma glucose is halved by 24 hours starvation, indicating up regulation.[18] Despite these adaptations, glucose transport at the blood-brain barrier is insensitive to insulin. Part of of the adjustment of glucose transport to the metabolic needs of the brain may depend on vascular responses (reviewed by Bradbury[4]), but the bulk of the modulation during hypo—and hyperglycaemia seems to be due to changes in the amount of available glucose transporter.

In the living rat, monocarboxylic acid transport into brain is potently regulated in relation to the concentration of ketone bodies in blood. Thus, the capacity of the system is greatly increased after several days starvation and during suckling in the baby rat when the milk diet is rich in fat.[19] The activity of the transporter is suppressed after portacaval anastomosis when the ability of the liver to produce ketone bodies is severely impaired.[20]

Retinal and brain capillaries

The retina is developmentally part of the brain and the neurones within it are structurally and functionally similar to those in the brain. Palm[22] repeated the experiments of Schnaudigel (1913) and confirmed that retina, optic nerve, lens and vitreous body did not take up trypan blue from blood. Whilst such experiments confirm the presence of a barrier in the retinal vessels, similar in this respect to the blood-brain barrier, 'the obstacle to the penetration of the dye cannot lie in the vessel wall alone'. The further barrier is, of course, the tight pigment epithelium which restricts solutes leaving the fenestrated capillaries of the choroid from entering the retina.

The permeability properties of the retinal vessels are more difficult to measure than those of the brain. This is partly because of the alternative transport route through the pigment epithelium but also because of the delicate nature of the retina and its ready contamination by radio-activity in the vascular choroid during studies using radiotracer in

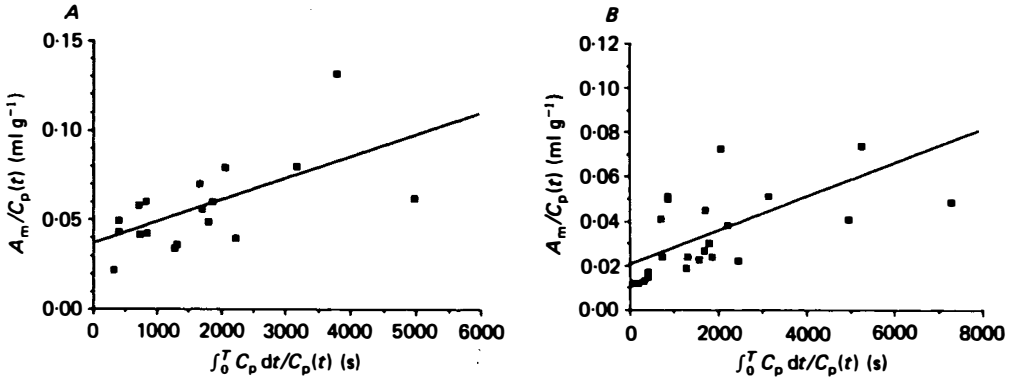

Fig. 1 *Tissue plasma concentrations of ^{14}C-mannitol in retina (A) and in occipital cortex (B) plotted against a function of time during uptake of the radiotracer for blood in the rat. The slope of the line is the permeability-surface area product and the intercept V, the rapidly equilibrating space, i.e. largely residual blood plasma in the tissue. From Lightman* et al.[22]

the blood. A number of studies have detected no ultrastructural or functional differences between retinal and brain capillaries. The permeabilities of the blood-retinal and blood-brain barriers are nearly identical both with respect to mannitol and to sucrose[22] (Fig. 1). These experiments indicate a high resistance of the pigment epithelium, as well as of the actual capillaries.

Specific transport mechanisms also appear to be similar in both types of capillary. Betz *et al*[23] have examined hexose transport into endothelial cells cultured from bovine retina. Uptake of labelled 3-O-methyl-glucose showed inhibition due to cytochalasin B, phloretin and phlorizin, equivalent to that seen in brain capillaries. Uptakes of various labelled amino acids from blood into rat retina *in vivo* showed a similar pattern to that observed in rat brain.[24]

Capillaries within the retina are generally surrounded by glial processes as in the brain. Significance has been attributed to the fact that the tight capillaries in the young rabbit lie on the retina within the vitreous humour and are not surrounded by such processes.[25] An equivalent situation occurs in frog brain where the capillaries are also fully tight but not invested by glia.[26] It may be noted that in both cases the capillaries are effectively lying on a glial membrane. These are the internal limiting membrane of the retina, composed of processes of Müller cells and the external limiting lamina in the brain composed of astrocytic processes.

Contrasting sensitivities of blood-retinal and blood-brain barriers in experimental diabetes

Microvascular lesions giving rise to increased permeability are well recognised in both human diabetes and in experimental models. Vascular damage is particularly evident in the retinopathy occurring in chronic diabetes in man. In view of the similarities between brain and retinal capillaries, discussed above, the question arises as to whether vascular changes occur in cerebral microvessels in chronic diabetes. If brain vessels are unaffected, what is the mechanism which protects them but not retinal microvessels?

Certainly there is evidence of thickening of the perivascular basement lamina in brain in both human diabetes[27] and in experimental diabetes in the rat,[28] together with indirect evidence of functional disturbance in the blood-brain barrier in man.[29]

A recent study was set up to investigate the permeability of brain and other microvessels in the streptozotocin diabetic rat maintained for up to 14 months.[30,31,32] Diabetic and control rats were each sub-divided into two further groups, receiving or not receiving an inhibitor of aldose reductose (Ponelrestat, ICI Pharmaceuticals) in the diet. At three weeks, six to seven months and 13-14 months, the extravascular uptakes of radio-iodinated albumin and of ^{14}C-sucrose into retina and into different regions of brain were measured. In control rats at 13-14 months, sucrose permeability into different regions of brain

varied from 0.82×10^{-5} ml.g^{-1}. S^{-1} (hippocampus) to 1.24×10^{-5} ml.g^{-1}. S^{-1} (medulla oblongata). The sucrose permeability of the blood-retinal barrier was comparable at 0.91 ml.g^{-1}. S^{-1}. The permeability into optic nerve was somewhat higher at 1.53×10^{-5}. Extravascular uptake of albumin into all central nerve tissues was insignificant at one hour. Sucrose permeability into retina was increased by 240% after 13-14 months of diabetes but was unaffected at three weeks and six to seven months. Uptake of neither sucrose nor albumin into brain was influenced by diabetes of any duration, except that extravascular uptake of albumin into the hypothalamic piece of brain, containing the high permeability median eminence, was raised. The aldose reductase inhibitor had no influence in preventing or mitigating the diabetes-induced permeability increase in retina and hypothalamus. A similar stability of the vascular barrier in brain, apart from the hypothalamus, has been reported for up to four weeks' diabetes in the rat.[33]

These results clearly confirm that the sucrose permeability of cerebral microvessels is in general more resistant to hyperglycaemia than those of the retina. The reason is less clear. The glucose concentration in cerebral extracellular fluids is < 50% of that in blood plasma and is probably kept low in hyperglycaemia by down regulation of the transcapillary transport as described above. Glucose in retina may go higher in diabetes because of the alternative route of entry through the pigment epithelium. Sorbitol in brain is less than in retina and in contrast to the situation in retina does not increase in diabetes. These observations would on their own be compatible with sorbitol being the agent causing microvascular damage in diabetes, but such an hypothesis is quite incompatible with the inability of the aldose reductase inhibitor to prevent raised sucrose permeability in the retina despite causing a big reduction in sorbitol in this tissue.[32]

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