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The blood–brain barrier in diabetes mellitus: A critical review of clinical and experimental findings

Evangelia Tolia, Ioannis P. Fouyas, Paul A.T. Kelly, Ian R. Whittle*

Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland, United Kingdom

Abstract. Although dysfunction of blood-retinal barrier in diabetes is well established, the role of the blood-brain barrier (BBB) in diabetic cerebral pathophysiology is unclear. A critical appraisal of the literature revealed many contradictory findings in both experimental and human studies. There is consistent evidence that the diabetic capillary basement membrane is thickened. However evidence of permeability dysfunction is limited, with the BBB reported as either normal (IgG, sucrose, cytochrome *c*, Horseradish peroxidase, EDTA, α-AIB) or variably leaky (albumin, inulin, Gd-DTPA). Evidence for dysregulation in blood-brain glucose transport, and the GLUT-1 transporter is inconsistent. Abnormal endothelial transport of choline, basic and neutral amino acids have been reported. Abnormalities have been identified in the tight junctional protein Occludin, but not in ZO-1. A difficulty with much of the experimental work in rodents is that different models have been used, numbers have often been small, methods variable and experimental studies performed after variable periods of diabetes or 'control' treatment. No studies have addressed dysfunction in efflux proteins such as P-glycoprotein or the organic cationic or anionic transporter families. Pathophysiological studies in humans, which do not support dysfunction of blood-brain glucose transport, are confounded by age, smoking, hypertension, type and duration of diabetes, quality of glycaemic control, and cerebrovascular disease. Future experimental and clinical studies with well defined controls and recorded co-morbidity, and utilisation of genomic and proteomic methodologies will help clarify the structural and functional changes in the diabetic BBB, and their contribution to neurological and cognitive dysfunction. © 2005 Elsevier B.V. All rights reserved.

Keywords: Blood–brain barrier (BBB); Diabetes; Basement membrane; Permeability; Transporter

* Corresponding author. Tel.: +44 0 131 537 2103; fax: +44 0 131 537 2561.
E-mail address: irw@skull.dcn.ed.ac.uk (I R. Whittle).

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1. Introduction

Diabetes mellitus (DM) is a metabolic, multi-system disorder associated with structural and functional alterations in a number of organs and dysregulation of various aspects of glucose, lipid and protein metabolism. Amongst the CNS complications of diabetes are a predisposition to cerebrovascular accidents, a range of ocular pathology, cognitive deficits, increased risk of dementia, impaired learning and memory and problem solving, as well as impairments in mental and motor speed and changes in mood and pain perception. Children may also experience profound brain oedema as a complication of diabetic ketoacidosis. The aetiology of these structural and functional disorders is multifactorial and includes accelerated atherosclerosis, hypertension, changes in blood viscosity, dysfunction of cerebral blood flow regulation, hyperosmolar insult and severe hypo- and hyperglycaemia.

The aetiology of brain dysfunction in diabetes is probably primarily related to small vessel disease however it is also possible that abnormalities in the structure and function of the blood-brain barrier (BBB) could contribute. The recent finding that diabetic patients appear to have a "leakier" BBB, is important since leukoaraiosis may be associated with cognitive impairment and increases the risk of dementia. Some structural similarities between the BBB and the blood-retinal barriers (BRB) and the fact that disruption of the BRB in diabetes causes severe ocular pathology is further evidence for the hypothesis that compromised BBB integrity could be, at least partly, responsible for aspects of diabetic brain pathology. The aims of this paper are therefore to: 1) briefly describe the normal structure of the BBB; 2) review publications concerning altered BBB function and structure in diabetes (in both animal models and humans); and 3) critically appraise these findings.

1.1. The normal BBB

The BBB is a diffusion barrier, which impedes influx of most compounds from blood to brain (see review by Ballabh et al. [1]). Its role is to maintain a homeostatic environment for neurons to function effectively and to exclude potentially toxic substances. The cellular elements of the BBB are: 1) endothelial cells, 2) astrocytic end-feet and 3) pericytes. The endothelium and pericytes have an abluminal basement membrane.

The BBB endothelial cells are characterised by the absence of fenestrations, extensive tight junctions (TJ) and sparse pinocytic vesicular transport. Endothelial cells are interconnected by the junction complex that comprises the TJs and the adherens junctions (AJ). TJs consist of three integral membrane proteins, namely, claudin, occludin, and junction adhesion molecules (JAM), and a number of cytoplasmic accessory proteins such as ZO-1, ZO-2, ZO-3, and cingulin. These cytoplasmic proteins link membrane proteins to actin (the primary cytoskeleton protein) in order to maintain the structural and functional integrity of the endothelium.

Pericytes appear to play a key role in angiogenesis, structural integrity and differentiation of the vessel, and formation of endothelial TJ. In an in vitro culture model pericytes stabilised the capillary-like structure formed by endothelial cells co-cultured with astrocytes by preventing apoptosis of the endothelium. Astrocytic end-feet tightly ensheath the pericytes and endothelium vessel wall and release trophic factors that are critical for the induction and maintenance of the BBB.

Compounds cross the BBB in several ways. These are by: 1) passive diffusion, particularly for lipid-soluble substances; 2) facilitative and energy-dependent receptor-mediated transporters, e.g., transferrin receptor, LDL-receptor; 3) Carrier mediated transporters which provide essential brain nutrients, e.g., GLUT-1 glucose, CAT-1 basic amino acids, LAT-1 neutral amino acids, EAAT-1 acidic amino acids; and 4) absorptive trancytosis, e.g., albumin. Additionally there are important mechanisms that efflux compounds from the endothelial membrane and basolateral membrane. Such well described proteins are P-glycoprotein (MDR-1), BCRP, OAT and OCT families.

2. Systematic review

Web-based tools, namely Pubmed and Science Direct, were used to conduct a systematic review of the literature. The search parameters set were: diabetes, blood-brain barrier, normal function and structure, and abnormalities.

2.1. Animal studies

2.1.1. Changes in permeability

Many of the findings summarised in Table 1 appear contradictory. For example, using immunocytochemical techniques Stauber et al. [2] found that albumin (but not IgG or C3) enters the cerebral cortex 2 weeks after the onset of streptozotocin-induced (STZ) diabetes. This appears paradoxical since albumin is a much larger molecule than, for example, sucrose whose permeability is not affected by diabetes. Increased transport of albumin could, however, be secondary to enhanced uptake of glycosylated albumin and this can be independent of alterations in the permeability of the BBB. This is supported by the finding that non-enzymatic glycosylation of serum albumin (as it occurs in DM) results in its increased micropinocytic up-take by endothelial cells. In studies with intravital microscopy using fluorescein-labelled albumin, diabetes was not associated with increased

Table 1
A summary of reports of changes in the permeability of several substances across the BBB in diabetic rats. Modified from Horani and Mooradian [4]

Compound	M.W. ($\times 10^3$)	Permeability	Duration of diabetes (weeks)	Reference
Albumin	67	Increased/un-altered	2	[11,33]
IgG	150	Unaltered	2	[11]
C3	80–110	Unaltered	2	[11]
Horseradish peroxidase	40	Unaltered	4	[12]
Cytochrome *c*	12	Unaltered	4–20	[34]
Inulin	4	Increased/un-altered	4 (but not 2)* and only in 3/12 regions	[12]
Sucrose	0.342	Unaltered	4–20	[13]
EDTA	0.372	Unaltered	2	[14]
α-aminoisobutyric acid	0.104	Unaltered	8	[7]

* Rats after 4 (but not 2) weeks of experimental diabetes had larger inulin spaces in certain areas of the cerebrum including the mediobasal and mediodorsal hypothalamus and the periaqueductal gray [5]. Horseradish peroxidase, on the other hand, remained confined to CNS areas devoid of BBB even when allowed to circulate for 75 min (versus 15 min that inulin was allowed to circulate) prior to killing.

permeability of the BBB and the diabetic rats did not appear to be more susceptible to BBB disruption with acute hypertension [3]. Again, results should be interpreted with caution since extravasation of tracer substances through the BBB is hard to quantify using intravital microscopy. Overall, the barrier properties of the BBB seem not to be grossly disturbed in STZ-treated rodents.

An explanation for these different findings may be related to the inherent problems of the STZ model. This rodent model is essentially a model of uncontrolled hyperglycemia, with limited tendency to ketoacidosis and animals survive without insulin. However there is considerable cachexia in this model. Additionally many studies have small numbers, do not report on animal morbity or mortality, some are performed using anesthetic agents, and there are variable periods after STZ before the study is performed. If the STZ model is considered akin to Type I human diabetes mellitus then certain BB substrain provide a model of type II diabetes. Further experimental studies on a more robust model such as the BB rat strains that spontaneously develops diabetes at specific ages may clarify the situation. In our own experimental work using the latter model (BB/E rats, aged 18–36 weeks, insulin dependent diabetes 8–20 weeks), we have identified widespread decreases in regional cerebral blood flow (rCBF) that range from 10–30%. However there was no abnormality in the BBB to alpha-aminoisobutyric acid, even after experimental intracerebral hemorrhage [6,7]. The decreased rCBF appears partly related to pertubations in eNOS vasoregulation and also perhaps the increased blood viscocity in the diabetics. There was also no thickening of the BM using electron microscopy in this model.

2.1.2. Structural and molecular changes

These involve the BM, astrocytic foot processes and components of the TJ such as occludin and ZO-1. In studies using STZ-induced diabetic animals, increased capillary BM thickening is a consistent finding. Using electron microscopy, Junker et al. [8] studied the BM of vessels in the hypothalamic arcuate nucleus and the occipital and frontal cortices in normotensive (WKY) and hypertensive (SHR) rats, 4 and 8 months after STZ. Although in the control group hypertension alone did induce BM thickening, they concluded that at both 4 and 8 months the BM was thicker in the diabetic compared to control rats and that hypertension combined with diabetes enhanced BM thickening in the frontal and occipital cortices but not in the hypothalamus. However in the hypothalamus they did find that hypertensive diabetic animals showed evidence of a degeneration of hypothalamic and cortical pericytes.

In contrast, Bouchard et al. [9] studied the BBB using EM and quantitative immunocytochemistry. They found that the morphological features of the capillary endothelial cells looked similar in control and diabetic rats (STZ induced, studied at 4–5 and 8–13 months). In both groups the endothelium was of the continuous type surrounded by a well-defined BM and within the cytoplasm of the endothelial cell the usual organelles were found: nucleus, RER, mitochondria and Golgi apparatus and plasmalemmal vesicles. The tight-junctional complexes between endothelial cells also remained intact. The last two observations are in particular in agreement with McCuskey and McCuskey [10]. However, the thickness of BM did increase in the diabetic rats, and this was related directly to the duration of diabetes. Furthermore, the

density of plasmalemmal vesicles (involved in transcytosis) almost doubled in the diabetic group and circulating albumin was preferentially associated with these vesicles. The increase in vesicle number could account for the apparent alteration in BBB permeability seen in diabetes. Accordingly, the amount of exogenous albumin taken up was higher in the diabetic animals when exogenous albumin was injected intravenously 15 min before the animal was sacrificed and then detected by protein A-gold immunocytochemical technique.

These results indicate that transport of albumin via vesicular structures undergoes changes during hyperglycaemia. Using specific anti-rat albumin, the authors found higher levels of endogenous albumin at the level of endothelial cells and their basement membranes in the diabetic condition. Interestingly, both exogenous and endogenous albumin in diabetic (but also older animals) were found to be closer to the astrocytic or pericytic site of the BM, indicating a diabetes-induced modification in the restrictive characteristics of the brain capillary BM. In young, normoglycaemic animals albumin vesicles were retained on the endothelial side of the BM, suggesting that similar to the glomerular wall, the BM of brain capillaries must contribute to the restricted passage of proteins across the capillary wall. Using in vivo and electron microscopic studies the astrocytic end-feet were noted to be swollen and contained mitochondria having longitudinal rearrangement of their cristae [10] in STZ-induced diabetic mice (from 2 weeks to 12 months). Since astrocytic end-feet are actively involved in the maintenance of the BBB, and in extracranial capillaries thickening of the BM is associated with decreased anionic site density, such abnormalities are likely to affect both barrier and transport properties.

Chehade et al. [11] carried out Western and Northern blot analyses to measure the steady-state level of occludin and ZO-1 proteins and mRNA levels in cerebral tissue of STZ-induced diabetic rats, insulin-treated diabetic rats and vehicle-injected control rats. The cerebral occludin content in untreated diabetic rats was significantly reduced compared to insulin-treated or control rats. The ZO-1 content, however, was not altered and neither were the levels of mRNA encoding the two proteins. This suggests that diabetes affects the translation of the occludin mRNA. Similar changes in occludin and ZO-1 levels are also known to be associated with ageing.

2.1.3. Changes in BBB-specific transport: Glucose

A number of studies employing a variety of methodologies have examined BBB glucose transport in hyperglycaemia. Some of these have reported depression [12–15], others reported no change [16,17], and yet others have found evidence of increased transport [18]. Gjedde and Crone were the first to study the effects of hyperglycaemia on the transport of glucose across the BBB [12]. They estimated glucose transport capacity by measuring the extraction of labelled glucose using an intravenous infusion paradigm in anaesthetized rats which allowed arterial plasma glucose concentration (C_a) to be varied over a wide range. While cerebral blood flow was found to be unaltered in chronic hyperglycaemia, suggesting that cerebrovascular surface area remained constant, the coefficients describing glucose transfer were 28% lower in the STZ-treated rats. Based on these findings the authors concluded that BBB glucose transport was downregulated in chronic hyperglycaemia. However the validity of this study is difficult

to assess given that the distribution of errors, weighting scheme and statistical analysis used were not discussed. Nonetheless the findings of this study were subsequently confirmed by McCall et al. [13], who found a 30% reduction at 2 day post STZ, and also by Mooradian and Morin [14] who used a different technique. At later time points, 8 days post-STZ, extraction fractions of a tracer bolus (3-O-methyl glucose), estimated by the brain uptake index (BUI) technique, were reduced by almost 70% [13]. Assuming unaltered cerebral blood flow the decreased BUI in hyperglycaemia was interpreted as reduced hexose transport. Reduced BBB glucose transport could be an adaptive change to protect the CNS from long-term glucotoxicity.

The two main factors that affect the interpretation of BUI measurements are the uncertain concentration of unlabeled substrate in the bolus as it traverses the vascular bed, and the unknown cerebral perfusion rate during the passage of the tracer bolus. In these studies, glucose concentration of the injectate was assumed to remain constant during its transit from the injection site in the carotid to the cerebral capillaries. There is also uncertainty regarding the precise vascular fluid that flows during the bolus passage. BUI values are dependent on flow, i.e., assumed to be unchanged in diabetes, whereas there is considerable evidence of reduced CBF. Additionally endothelial pathophysiology, and in particular altered bioavailability of endothelial nitric oxide, might differentially effect vascular flow responses to intracarotid bolus injections in the diabetic and control groups.

To avoid some of the uncertainties inherent in the BUI method, an in situ internal carotid artery infusion technique was employed to evaluate BBB glucose transport (6–7 days post-STZ, serum glucose=25 mM) versus normoglycaemic rats [15]. Their method allowed for simultaneous estimation of glucose extraction and blood flow in the hemisphere ipsilateral to the carotid catheter, and calculation of the permeability/surface area (PS)-product. An electrolyte solution containing albumin and labelled diazepam was used as the infusate vehicle (which also included labelled glucose to calculate the clearance). STZ-treated rats had a 44% lower value for the calculated PS-product, but this was due to a concomitant 44% reduction in cerebral vascular fluid flow. It should be noted, however, that vascular fluid flows in normal rats in this study were much lower than the values reported in the original description of this technique. This could be due to the haemodynamic effect of albumin in the perfusate (not included in the original technique).

On the other hand, when a labelled glucose i.v. infusion technique was used to estimate regional PS-products for glucose transfer in awake rats, there were no significant differences between chronically hyperglycaemic (1 and 3 weeks post-STZ) and acutely hyperglycaemic rats [16]. This suggested no change in BBB glucose transport capacity in chronic hyperglycaemia. Harik and LaManna [17] reached a similar conclusion in a study using the dual tracer, intra-atrial bolus technique to measure, simultaneously, extraction and CBF. However, systemic administration studies are potentially vulnerable to data variability, changes in nonsaturable diffusion, and inaccuracies in the estimates of PS products or BBB glucose influx rate. For example, in the study by Duckrow [16] decreases in the PS product of up to 17% did not reach statistical significance because of data scatter.

In a more recent study Simpson et al. [19] made a quantitative assessment of alterations in BBB GLUT-1 distribution in vivo, in chronic hyperglycaemia, using the impermeant

photolabel ATB-BMPA to specifically measure the concentration of GLUT-1 on the luminal surface of the endothelial cells. They combined this methodology with studies of glucose uptake using the in situ internal carotid perfusion technique and determination of total BBB GLUT-1 concentration (Western blot analysis) and analysis of GLUT-1 mRNA expression (in situ hybridization) in isolated cerebral microvessels of diabetic rats. They found no difference in glucose uptake between diabetic and control animals. The lack of effect of diabetes on brain glucose transport was further demonstrated by measurement of total GLUT-1 transporter and accessible luminal transporters. Neither parameter changed in diabetic animals. Pardridge et al. [15], however, reported a reduced GLUT-1 concentration in cerebral microvessels of STZ-treated rats by using the same immunoblot assay. The levels of GLUT-1 mRNA localised to the BBB paradoxically increased by almost 100%. It is interesting that in a different model of diabetes, the hyperglycaemic *db/db* mouse [20], the same pattern of results was observed; i.e. the levels of GLUT-1 transporter were unaltered despite increases in GLUT-1 mRNA.

Using a modification of a double-label 2-deoxyglucose method, regional BBB glucose influx rate, glucose utilisation, and brain glucose concentrations were compared in normoglycaemic control versus STZ-treated (6–8 weeks) rats rendered acutely normoglycaemic by i.v. insulin [18]. The latter group had higher values for cerebral glucose metabolic rate, glucose influx rate and brain/tissue plasma ratios in most of the brain regions analysed, implying increased glucose transport in the STZ-treated rats. These results do not support the hypothesis that cerebral glycopenia is responsible for hypoglycemic symptoms when glucose is acutely normalised in human diabetics.

The effects of hyperglycaemia on the glucose transporter have also been investigated ex vivo, in isolated cerebral microvessels. In these studies, hyperglycaemic conditions were imposed in vivo and assays for the glucose transporter, using cytochalasin B (CB), were performed in isolated cerebral microvessels. CB is a competitive inhibitor of GLUT-1 and displaces D-glucose. Three studies that have used this technique have come up with conflicting results. In one study a 38–50% decrease in maximal CB binding was measured in several subcellular fractions of microvessels [21]. It was concluded that in chronically hyperglycaemic rats there is a reduction in the density of GLUT-1 at the BBB. A concern with this study is that the maximal CB values, even in controls, were much lower than those reported elsewhere in the literature. A subsequent study found a 30% increase in the maximal CB binding sites in fractions of cerebral microvessels isolated from hyperglycaemic rats, indicating up-regulation of GLUT-1 at the BBB [17]. To further complicate this matter a third investigation found no significant differences in the density of specific CB binding sites in brain microvessels of STZ-treated rats [14]. Again, there are certain problems with this method. Although CB is a good method for quantitation of glucose transporters it is not specific to GLUT-1 (CB binds to a number of facilitative glucose transporters). Additionally, a change in glucose transporter density in the cerebral microvessels does not necessarily mean a corresponding change in glucose transport at the BBB. The glucose transporter density is a relatively static value which changes only slowly in response to an altered homeostatic environment, whereas glucose transport is a dynamic state and may change on a moment-to-moment basis. Moreover, not all transporters that bind CB may be functional at the blood–brain interface; at least a portion of them may be located in intracellular organelles.

2.1.4. Changes in BBB transport: choline and amino acids

Mooradian [22] found that the BUI for choline (the substrate for the neurotransmitter acetylcholine), as well as the V_{max} for its transport was significantly reduced in STZ-treated mice, and alterations in brain choline appeared to be dependent on the duration of diabetes. Another study has shown reduced synthesis of acetylcholine in the rat striatum. It is not clear which represents the cause and which is the effect. Reduced BBB choline transport could, therefore, be an adaptive response to reduced acetylcholine synthesis or reduced utilisation of choline in cerebral tissue.

Using a continuous infusion method and quantitative autoradiography, Mans et al. [23] determined that PS and influx of phenylalanine, and several other basic amino acids (e.g. tryptophan, tyrosine and methionine), were reduced in diabetic rats. In contrast, PS and influx of neutral amino acids, including lysine, were markedly increased. These observations, however, might not necessarily reflect an alteration of the transport at the BBB level, it could for example be that serum concentrations of the amino acids decreased and increased, respectively, thus affecting the amount of substrate available for transport. Reduced influx could be responsible for low brain content of some essential amino acids, with possibly deleterious consequences for brain functions. McCall et al. [13] found that lysine extraction was unaltered in diabetic rats (enters the brain by a basic amino acid carrier) and so were the extractions of tyrosine and tryptophan, which enter the brain via the neutral amino acid carrier.

2.1.5. Pathophysiological changes of cerebral microvessels

Vascular abnormalities may contribute to dysfunction in the BBB, may impair delivery of essential nutrients to neuronal tissue, and predispose the animal to cerebral hypoxic injury during reduced perfusion pressure or poor ventilation. Histological changes in the cerebral microvessels in diabetes include reduced density of cortical capillaries and increased capillary BM thickening; arteriovenous shunting and paucity of cortical capillaries and calcium depositions in microvessel walls of diabetic animals [10]. In this context Mooradian and Smith [24] reported a significantly higher concentration of lipid peroxidation by-products, namely conjugated dienes, after 5 weeks of STZ-induced diabetes. The accumulation of conjugated dienes in cerebral microvessels in the absence of an alteration in the availability of substrates for peroxidation is consistent with the hypothesis that DM is a disease state that is associated with increased free radical activity. The same authors found that cholesterol, phospholipid and fatty acid composition were not significantly affected.

In addition to causing the accumulation of lipid peroxidation by-products, diabetes can also affect the protein composition of cerebral microvessels, which may also contribute to diabetes-related changes in the BBB. Using two-dimensional electrophoresis and computer-assisted densitometry, it was found that the level of glycosylation of cerebral microvessel protein mixture was significantly increased after five weeks of uncontrolled diabetes and that 1 out of the 25 proteins quantified was significantly altered [25]. The role of advanced glycosylation end products (AGE) in the pathophysiology of the diabetic capillary bed is not well understood.

2.2. Human studies

2.2.1. Permeability of BBB

Starr et al. [26] used MRI to investigate whether the BBB is compromised in diabetes and whether changes in BBB integrity underlie structural abnormalities. MRI of the brain was conducted in 10 men with NIDDM (aged 65–70 years) and ten age-matched non-diabetic controls. Subtracted net signal intensities of various brain regions were derived from T1-weighted images at different time points after gadolinium-DTPA (Gd-DTPA). The authors interpreted increased net signal intensity as extravasation of Gd-DTPA from the vascular space and, therefore, used it as a measure of BBB permeability. In several brain regions (and especially the basal ganglia), net Gd-DTPA signal intensity in the first 15 min after contrast administration was greater in diabetic individuals. Signal intensity was also increased in all individuals with leukoaraiosis but there was no positive correlation between increased signal intensity and diabetic individuals with leukoaraiosis. Although alternative explanations have been offered in a thoughtful editorial [27], the authors concluded that BBB permeability is increased in human diabetes.

In contrast to the in vivo imaging study Dai et al. [28] performed immunohistochemical staining on post-mortem prefrontal and temporal cortices of diabetic patients and controls, using specific antibodies against PAL-E, IgG and albumin. These proteins are considered markers for the vascular permeability status of the BBB. They found no difference in protein staining between diabetic and control brains. These results suggest that the BBB is well-maintained in diabetic patients.

2.2.2. Glucose transport

Although patient studies are much fewer in number, the inconsistent data on glucose transport from animal studies have not been repeated in studies of the human diabetic brain. None of 4 studies currently in the literature has detected any abnormality: Brooks et al. [29] found no significant difference in brain [^{11}C]3-O-methyl-glucose extraction in four patients with poorly controlled type I diabetes compared with four non-diabetic control subjects, each with plasma glucose levels of 3.8 mmol/l. There are certain limitations in the design of this study. Firstly, although the mean rate of blood-to-brain glucose transport was 18% lower in the diabetic patients; this relatively large difference did not reach statistical significance in their small group. Secondly, the behaviour of a glucose analogue, and not glucose, was studied. Interpretation of data obtained with a glucose analogue implicitly assumes that any change in BBB glucose transport in type I diabetes does not involve a change in affinity of the transporter for the analogue relative to glucose. Similarly, Gutniak et al. [30] found no significant difference in the blood-to-brain transport of a glucose analogue in six patients with well-controlled type I diabetes compared with 8 non-diabetic controls during euglycaemia and hypoglycaemia. However, the methodology, as in the previous study, may make the study prone to false-negative conclusions.

In a study using radiolabelled glucose and MRI, there was no difference in the calculated brain glucose concentration between well-controlled type I diabetic patients and controls. That method failed to provide direct measure of blood-to-brain glucose

transport or cerebral glucose metabolism. A study by Fanelli et al. [31] also showed no difference in glucose transport between diabetic and control subjects. Seven patients with poorly controlled type I diabetes were studied versus nine non-diabetic patients during hyperinsulinaemic, mildly hypoglycaemic glucose clamps. The authors determined the rates of blood-to-brain glucose transport and cerebral glucose metabolism with PET, using glucose (and not a glucose analogue as previous studies had done) and with ^{11}C glucose. CBF and cerebral blood volume were determined by intravenous $H_2^{15}O$ and inhaled $C^{15}O$, respectively (also by PET). At plateau plasma glucose concentrations of around 3.6 mmol/l, neither blood-to-brain glucose transport nor cerebral glucose metabolism were measurably altered in people with poorly controlled type I diabetes.

2.2.3. Histological abnormalities

Johnson et al. [32] studied the thickness of the cerebral cortical capillary basement membrane in frontal and occipital lobes from 21 diabetics and 16 non-diabetics and, as in animal studies, found that those from diabetics were significantly thicker.

3. Summary

In terms of increased permeability, the BBB appears to be less susceptible to the effects of uncontrolled hyperglycaemia than the BRB. The precise reason for this difference is unknown. It may result from the influence of the surrounding tissue or with the richness of cerebral microvessels in antioxidant enzymes or may be the result of down-regulation of glucose transport, thereby protecting the CNS against metabolic changes induced by hyperglycaemia. Problems may also arise from the use of the STZ model. It is likely that the advent of transgenic models, and in particular conditional transgenics, will greatly advance the usefulness of animal studies in modelling the human condition. Application of genomic, e.g. microarray technology, and proteomics methods in such models, as well as study of efflux proteins and organic cationionic and anionic transporters, may help identify diabetic induced changes.

Overall there is no strong experimental evidence to suggest that diabetes may cause brain parenchymal pathology by affecting the permeability of the BBB. Diabetes does appear to mimic the ageing process and thus may accelerate senility related brain dysfunction. Similarities between diabetes and ageing include: 1) The BM is thicker in diabetic and also older animals. Increased BM thickness might result in restricted transport of essential substances and brain dysfunction; 2) Albumin (both exogenous and endogenous) is localised closer to the pericytic/astrocytic side of the BM in diabetic and older, non-diabetic animals. This indicates an alteration in the restrictive characteristics of the brain capillary BM; 3) Changes in cerebral occludin content were similar in aging and diabetic processes.

Most human studies suggest that diabetes does not affect glucose transport, permeability or BBB structure. Starr's study was the first study to report positive results, however whole-brain imaging techniques may not have sufficient resolution to detect localised areas of microvascular leakage characteristic of diabetic microangiopathy. What

make human studies very difficult to interpret is the complexity of processes that cause CNS disease in diabetes and the influence of other variables—e.g. type of diabetes, duration of diabetes, gender, degree of metabolic control, hyperlipidaemia, hypertension, smoking and concomitant drug therapy—all of which could potentially affect BBB function.

References

[1] P. Ballabh, A. Braun, M. Nedergaard, The blood-brain barrier: an overview. Structure, regulation and clinical implications, Neurobiol. Dis. 16 (2004) 1–13.
[2] W.T. Stauber, S.H. Ong, R.S. McCuskey, Selective extravascular escape of albumin into the cerebral cortex of the diabetic rat, Diabetes 30 (6) (1981) 500–503.
[3] W.G. Mayhan, L.K. Simmons, G.M. Sharpe, Mechanism of impaired responses of cerebral arterioles during diabetes mellitus, Am. J. Physiol. 260 (1991) H319–H326.
[4] M.H. Horani, A.D. Mooradian, Effect of diabetes on the blood brain barrier, Curr. Pharm. Des. 9 (2003) 833–840.
[5] M. Lorenzi, et al., Studies on the permeability of the blood-brain barrier in experimental diabetes, Diabetologia 29 (1986) 58–62.
[6] I.P. Fouyas, et al., Cerebrovascular responsiveness to *N*-nitro-l-arginine methyl ester in spontaneously diabetic rats, Br. J. Pharmacol. 118 (1996) 243–248.
[7] I.P. Fouyas, et al., Cerebrovascular responses to pathophysiological insult in diabetic rats, J. Clin. Neurosci. 10 (1) (2003 Jan.) 88–91.
[8] U. Junker, et al., Basement membrane of the hypothalamus and cortex capillaries from normotensive and spontaneously hypertensive rats with streptozotocin-induced diabetes, Acta Neuropathol. 65 (3–4) (1985) 202–208.
[9] P. Bouchard, L.D. Ghitescu, M. Bendayan, Morpho-functional studies of the blood-brain barrier in streptozotocin-induced diabetic rats, Diabetologia 45 (2002) 1017–1025.
[10] P.A. McCuskey, R.S. McCuskey, In vivo and electron microscopic study of the development of cerebral diabetic microangiography, Microcirc. Endothel. Lymphat. 1 (1984) 221–244.
[11] J.M. Chehade, M.J. Haas, A.D. Mooradian, Diabetes-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1) expression, Neurochem. Res. 27 (2002) 249–252.
[12] A. Gjedde, C. Crone, Blood-brain glucose transfer: repression in chronic hyperglycemia, Science 214 (1981) 456–457.
[13] A.L. McCall, W.R. Millington, R.J. Wurtman, Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus, PNAS 79 (1982) 5406–5410.
[14] A.D. Mooradian, A.M. Morin, Brain uptake of glucose in diabetes mellitus. The role of glucose transporters, Am. J. Med. Sci. 301 (1991) 173–177.
[15] W.M. Pardridge, D. Triguero, C.R. Farrell, Down-regulation of blood-brain barrier glucose transporter in experimental diabetes, Diabetes 39 (1990) 1040–1044.
[16] R.B. Duckrow, Glucose transfer into rat brain during acute and chronic hyperglycaemia, Metab. Brain Dis. 3 (1988) 201–209.
[17] S.I. Harik, J.C. LaManna, Vascular perfusion and blood-brain glucose transport in acute and chronic hyperglycaemia, J. Neurochem. 51 (1988) 1924–1929.
[18] D.A. Pelligrino, M.D. Lipa, R.F. Albrecht, Regional blood-brain glucose transfer and glucose utilization in chronically hyperglycemic, diabetic rats following acute glycemic normalisation, J. Cereb. Blood Flow Metab. 10 (1990) 774–780.
[19] I.A. Simpson, et al., Blood-brain barrier glucose transporter. Effects of hypo- and hyperglycaemia revisited, J. Neurochem. 72 (1999) 238–247.
[20] S.J. Vannucci, M. Gibbs, I.A. Simpson, Glucose utilisation and glucose transporter proteins GLUT-1 and GLUT-3 in brains of diabetic (*db/db*) mice, Am. J. Physiol. 272 (1997) E267–E274.
[21] S. Matthaei, R. Horuk, J.M. Olefsky, Blood-brain glucose transfer in diabetes mellitus. Decreased number of glucose transporters at the blood-brain barrier, Diabetes 35 (1986) 1181–1184.

[22] A.D. Mooradian, Blood-brain barrier choline transport is reduced in diabetic rats, Diabetes 36 (10) (1987) 1094–1097.
[23] A.M. Mans, et al., Regional amino acid transport into brain during diabetes. Effect of plasma amino acids, Am. J. Physiol. 253 (1987) E575–E583.
[24] A.D. Mooradian, T.L. Smith, The effect of experimentally induced diabetes mellitus on the lipid order and composition of rat cerebral microvessels, Neurosci. Lett. 145 (1992) 145–148.
[25] A.D. Mooradian, et al., Diabetes-related changes in the protein composition of rat cerebral microvessels, Neurochem. Res. 19 (1994) 123–128.
[26] J.M. Starr, et al., Increased blood-brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging, J. Neurol. Neurosurg. Psychiatry 74 (2003) 70–76.
[27] A.K. Kumagai, Diabetes and the blood-brain barrier, Lancet Neurology 2 (2003) 209.
[28] J. Dai, G.F.J.M. Vrensen, R.O. Schlingemann, Blood-brain barrier integrity is unaltered in human brain cortex with diabetes mellitus, Brain Res. 954 (2002) 311–316.
[29] D.J. Brooks, et al., Regional cerebral glucose transport in insulin-dependent diabetic patients using [11C]3-O-methyl-D-glucose and positron emission tomography, J. Cereb. Blood Flow Metab. 6 (1986) 240–244.
[30] M. Gutniak, et al., D-[U-^{11}C]glucose uptake and metabolism in the brain of insulin-dependent diabetic subjects, Am. J. Physiol. 258 (1990) E805–E818.
[31] C.G. Fanelli, et al., Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes, Diabetes 47 (1998) 1444–1450.
[32] P.C. Johnson, K. Brenda, E. Meezan, Thickened cerebral cortical capillary basement membranes in diabetics, Arch. Pathol. Lab. Med. 106 (5) (1982 May) 214–217.
[33] M.W.B. Bradbury, et al., Permeability of blood-brain and blood nerve barriers in experimental diabetes mellitus in the anaesthetized rat, Exp. Physiol. 76 (1991) 887–898.
[34] J. Jakobsen, L. Malmgren, Y. Olsson, Permeability of the blood nerve barrier in the streptozotocin diabetic rat, Exp. Neurol. 60 (1978) 277–285

Discussion

Tsuji
I know from studies evaluating blood-brain barrier in spontaneous hypertensive rats and in rats made diabetic by treatment with streptozotocin that in both cases there is a reduction in choline levels, so if one uses SHRs made diabetic there should be a clear reduction of choline concentrations. Do you have any data?

Whittle
We haven't done anything on this. There are studies looking at choline levels in other strains of rats in the basal ganglia, and the levels are decreased compared with normal animals.

De Boer
Is the thickening of the basement membrane that occurs due to the accumulation of non-basement materials or is it typical basement material?

Whittle
I tried to find the answer to that, but it is not obvious in the literature. But it would appear that maybe a glycosylated end-product. Actually the amount of proteoglycans has gone down. So I think, from what I have read, that the biochemical constitution is altered and that has lead to the charge difference as well.

Smith

I think your comment about the transporters is a good one because, if I recall correctly, Tom Davis' group has got some data showing changes at least in some ion transporters at the choroid plexus in diabetes. That may suggest that some of the other transporters may be similarly affected.