Glucose Transport in Brain and Retina: Implications in the Management and Complications of Diabetes

Arno K. Kumagai*

Department of Internal Medicine, Michigan Diabetes Research and Training Center, University of Michigan Medical School, Ann Arbor, MI, USA

*Correspondence to: Department of Internal Medicine, 5570 MSRB-2, University of Michigan Medical School, Ann Arbor, MI 48109–0678, USA
E-mail: akumagai@umich.edu

Summary
Neural tissue is entirely dependent on glucose for normal metabolic activity. Since glucose stores in the brain and retina are negligible compared to glucose demand, metabolism in these tissues is dependent upon adequate glucose delivery from the systemic circulation. In the brain, the critical interface for glucose transport is at the brain capillary endothelial cells which comprise the blood–brain barrier (BBB). In the retina, transport occurs across the retinal capillary endothelial cells of the inner blood–retinal barrier (BRB) and the retinal pigment epithelium of the outer BRB. Because glucose transport across these barriers is mediated exclusively by the sodium-independent glucose transporter GLUT1, changes in endothelial glucose transport and GLUT1 abundance in the barriers of the brain and retina may have profound consequences on glucose delivery to these tissues and major implications in the development of two major diabetic complications, namely insulin-induced hypoglycemia and diabetic retinopathy. This review discusses the regulation of brain and retinal glucose transport and glucose transporter expression and considers the role of changes in glucose transporter expression in the development of two of the most devastating complications of longstanding diabetes mellitus and its management. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords blood–brain barrier; blood–retinal barrier; glucose transport; hypoglycemia; diabetic retinopathy; diabetes

Introduction
Glucose is an essential metabolic substrate of all mammalian cells. Neural tissue in particular, i.e. the brain and retina, is entirely dependent on glucose under normal physiological conditions; however, access of neural tissues to a steady supply of this nutrient is limited by two factors. First, glycogen stores within neural tissue are either non-existent (e.g. brain) or inadequate to meet basal metabolic demands (e.g. retina). This relative lack of local carbohydrate stores results in a total dependence of neural tissue on delivery of glucose from the circulation [1,2]. Second, each of these tissues possesses a barrier between itself and its blood supply. In the brain, the presence of tight junctions and a lack of fenestrations and pinocytic activity characterize the microvascular endothelial cells that comprise the BBB [3]. In the retina, two barriers exist: the retinal capillary endothelial cells of the inner BRB, a microvascular bed which is highly similar to that in the brain, and the retinal pigment epithelium, a single layer of epithelial cells connected by tight junctions which is located between the photoreceptor elements and the choroid and is known as the outer BRB [4]. The presence of these barriers in
neural tissue prevents the passive diffusion of glucose and other blood-borne nutrients into the interstitial space surrounding neural cells. Instead, glucose entry is mediated by a specific, saturable, facilitated transport process involving members of the sodium-independent glucose transporter family (Table 1) [5,6]. The purpose of the present review is to discuss glucose transport and glucose transporter expression in the brain and retina and their respective barriers. The review also discusses the possible implications that changes in brain and retinal glucose transport may have in the pathogenesis of a complication associated with the treatment of diabetes (iatrogenic hypoglycemia) and of one of the sequelae of long-term hyperglycemia (diabetic retinopathy).

Five functional isoforms of the GLUT family have been identified and are numbered in the order of their characterization. The various glucose transporter isoforms demonstrate, at least to a certain degree, tissue-specific localization [5,6]. GLUT1, the first isoform cloned [7], is expressed in high density in the membranes of human erythrocytes and is characteristically expressed in blood–tissue barriers, such as the BBB, the blood–ocular barriers and the blood–placental and blood–testis barriers [2,8,9]. GLUT2 is expressed predominantly in the liver and in pancreatic β-cells [10], where, functionally coupled to hexokinase, it plays a role in sensing blood glucose levels. GLUT3, originally isolated from a fetal muscle cell line [11], has been identified as the primary glucose transporter in neural elements of the brain [12]. GLUT4 is expressed in high abundance in skeletal muscle and fat [13,14], where it mediates insulin-sensitive glucose transport, and GLUT5 is a fructose transporter that is expressed predominantly in intestinal epithelium [15]. The glucose transporter isoforms share 39–65% amino acid sequence identity [5], and sequence comparisons between GLUT1 of various species reveal an extremely high degree of conservation (>95%) [16], thus emphasizing the critical and universal importance of this transporter in cellular metabolism. Hydrophobicity sequence analysis suggests that the GLUTs are arranged in 12 membrane-spanning regions [7]. In the case of GLUT1, there is a single N-linked glycosylation site at Asp45 in the first extracellular loop [7]. Differential glycosylation of this residue accounts for the different molecular weights of GLUT1 in different cell types [17] and may play a role in the subcellular localization of GLUT1 [18,19]. The sodium-independent glucose transporters mediate transport of glucose, glucose analogs and other hexoses, with conformational specificity (i.e. transporting D- but not L-isomers) and different affinities for hexoses and glucose analogs. In addition, GLUT1 and GLUT3 have been demonstrated to be responsible for the transport of ascorbic acid [20], which, in brain capillary endothelia, is transported in its oxidized form [21].

### Glucose transport and glucose transporters in brain

Glucose transport from the blood into the cytosol of the neuronal elements of the brain occurs across several membranes in series. Glucose first enters the cytoplasm of the endothelial cells of the BBB by transport across the endothelial luminal membrane via GLUT1. Subsequent transport across the ablumenal membrane and into the interstitial space is also mediated by the GLUT1 isoform. Finally, glucose uptake into the cytosol of the neuronal cells occurs via the neuronal GLUT3 glucose transporter [22,23]. Cytochalasin-B binding studies suggest that the density of glucose transporters on the BBB is 2–3 times that on the neuronal cells [24]; however, because the surface area of the BBB is less than 0.1% of that of the neuronal elements of the brain, transport of glucose from blood into brain is limited by its transport across the BBB [1].

In studies using light microscopic immunohistochemistry and in situ hybridization of rat brain sections, GLUT1 is localized to the endothelia of the BBB [24]. Northern blotting analysis of bovine brain capillary total RNA [25] and rat brain poly(A+) mRNA [26] demonstrates a characteristic 2.8-kb band corresponding to the GLUT1 transcript, and GLUT1 mRNA is not detected in bovine and rat brain which has been depleted of vascular elements [25,26]. These observations have lent support to the hypothesis that GLUT1 is exclusively expressed in the endothelial cells of the BBB [24,25]. In contrast, GLUT1 is clearly detectable as a band of 45 kDa on Western blots of vascular-depleted brain protein, compared to the broad 54-kDa band seen in preparations of isolated brain capillaries [19,24,27]. The difference in molecular weight between ‘vascular’ and ‘non-vascular’ GLUT1 is due to differential glycosylation of the asparagine residue in the first extracellular loop of the transporter, with a larger carbohydrate side chain on the vascular form of GLUT1 [19,24]. Localization of ‘nonvascular’ GLUT1 has been attributed to glial cells: using in situ hybridization, Bondy and colleagues have reported in rat brain sections scattered signals for GLUT1 transcript which

### Table 1. Isoforms of the sodium-independent facilitative glucose transporter family and their characteristic tissue localization

<table>
<thead>
<tr>
<th>GLUT isoform</th>
<th>Tissue localization</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>Human erythrocyte, blood–brain barrier, blood–retinal barrier, blood–placental barrier</td>
<td>Characteristically expressed in cell types with barrier functions</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, pancreatic β-cell</td>
<td>Participates in pancreatic glucose sensing</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Brain</td>
<td>Major transporter in CNS neural tissue</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Fat, skeletal muscle, myocardial tissue</td>
<td>Insulin-sensitive</td>
</tr>
<tr>
<td>GLUT5</td>
<td>Intestinal epithelium</td>
<td>Fructose transporter</td>
</tr>
</tbody>
</table>

appear to localize to astrocytes [28], and in recent studies, McCall and co-workers have reported specific staining for GLUT1 in the parenchyma of normal rat brains which colocalizes with glial fibrillary acidic protein (GFAP), a specific marker of cells of glial origin [29]. Furthermore, Yu and co-workers have demonstrated GLUT1 immunoreactivity in rat brain glia and oligodendrocytes by electron microscopic immunohistochemistry [30]. Additional, indirect evidence of GLUT1 in brain glial cells is the abundant expression of GLUT1 protein and transcript in primary cultures of rat brain glial cells [31]. These latter data, however, must be interpreted with caution, since GLUT1 may be expressed in most cells in culture, even in cell types which do not express the protein in vivo [6]. Nonetheless, evidence from cytochalasin-B binding assays [32] and immunohistochemical [24,29] and in situ hybridization studies [24,28] suggest that GLUT1 is predominantly expressed on the BBB.

At a subcellular level, GLUT1 is localized to both the luminal and abluminal membranes of the endothelial cells of the rat BBB, and approximately 40% of total cellular GLUT1 in the rat BBB resides in the cytosol [33]. An asymmetrical distribution of GLUT1 between the luminal and abluminal membranes of the rat BBB has been documented, in which the ratio of luminal to abluminal transporters is approximately 1:4 [33], an observation which suggests that glucose transport at the BBB is limited at the luminal membrane. This pattern of asymmetrical distribution has been reported in the BBB of the developing rabbit as well [34]. Taken together, these data suggest that the luminal membrane of the endothelial cells represents a critical interface for glucose entry into brain: BBB glucose transport may be upregulated by either increased expression of GLUT1 or, in a more acute setting, by translocation of pre-existing cytosolic transporters to the luminal membrane [34].

As mentioned above, GLUT3 has been identified as the principal glucose transporter isoform in neural cells in brain. Gerhart and co-workers have used an antiserum directed against the carboxyl terminus of human GLUT3 to report the presence of GLUT3 in the canine BBB as well [35]; however, this observation has been challenged by others [27], who cite a lack of specificity between human and canine carboxyl terminus amino acid sequences of this transporter isoform. Based on numerous studies, a consensus has emerged that in the brain, GLUT3 is localized to the neuropil [27,36,37]. With regard to expression of other glucose transporter isoforms on the microvasculature of the brain, studies from other laboratories have reported the apparent localization of GLUT2 in the hypothalamus [38], and the insulin-sensitive transporter, GLUT4, in the BBB of the frontal cortex [39]. The presence of the latter isoform – albeit, at very low abundance – on the BBB is intriguing, since glucose uptake by the brain is thought to be insulin-insensitive [1]. BBB expression of GLUT5, the fructose transporter [40], and of a sodium-dependent glucose co-transporter similar to SGLT2 [41] have also been reported. Nonetheless, the extremely close agreement between the density of D-glucose-displaceable cytochalasin-B binding sites and immunoreactive GLUT1 abundance on isolated brain capillary preparations [42] provides evidence that glucose transport across the BBB is predominantly mediated by GLUT1.

Although glucose transport into whole brain has been extensively studied in both animal models [26,43–45] and human subjects [46], it is clear that there is a great deal of heterogeneity in brain glucose uptake and utilization in different brain regions [47–50]. Glucose transporter gene expression in the brain also demonstrates regional variation [51,52]. For example, GLUT1 is expressed in higher density in gray matter than white matter, and its expression is positively correlated with regional glucose utilization [52]. GLUT3 expression shows regional heterogeneity; however, unlike GLUT1, there is no correlation with regional glucose consumption [53]. Western blotting studies by Brant and co-workers using isoform-specific antisera demonstrate that GLUT2 is expressed at low levels throughout the rat brain, with the highest levels in the optic chiasm and pituitary. In contrast, GLUT4 expression is predominantly limited to the pituitary, hypothalamus and cerebellum [51]. (The apparent contradiction between an inability to detect GLUT4 in frontal cortex by Western blotting by Brant et al. [51] and the presence of GLUT4 in microvessel preparations reported by McCall et al. [39] may be explained by the extremely low abundance of GLUT4, in comparison to that of GLUT1, in the frontal cortex [39]).

Regulation of glucose transporter expression in brain

In the brain, GLUT1 expression is subject to developmental regulation with a decrease in immunoreactive GLUT1 in the immediate postnatal period, followed by a gradual increase in localization of GLUT1 to the BBB and an increase in its abundance to levels comparable to those in adults by 30–60 days after birth [42,54,55]. The gradual increase in BBB GLUT1 expression and decrease in parenchymal (i.e. nonvascular) GLUT1 correlate with the maturation of the BBB in the rat brain [56]. The neuronal glucose transporter GLUT3 also undergoes developmental regulation, which coincides with neuronal maturation [57].

In studies using bovine brain endothelial cell cultures, an in vitro model of the BBB, GLUT1 mRNA expression has been shown to be upregulated in response to glucose deprivation [58,59], phorbol esters and serum [60] and growth factors, including tumor necrosis factor-α (TNF-α) [61], brain-derived peptides [62] and glial-derived factors [63,64]. Of note, GLUT1-mediated glucose transport in brain capillary endothelial cells is not affected by insulin [65], an observation which supports the concept that BBB glucose transport is insulin-insensitive [1]. Upregulation of brain microvascular endothelial GLUT1 abundance in response to glucose deprivation appears to be regulated at a post-transcriptional level, since nuclear
run-on assays of bovine brain endothelial cells grown in media at low glucose concentrations demonstrate transcription rates which are similar to that of controls [59]. Actinomycin D studies of these cell cultures reveal a prolongation of the half-life of the GLUT1 transcript in glucose-deprived cell cultures, suggesting that the increase in GLUT1 mRNA (and by inference, in immunoreactive GLUT1 protein) during glucose deprivation occurs via stabilization of the GLUT1 transcript [59].

In animal models, BBB glucose transport and/or GLUT1 abundance has been shown to be modulated by streptozotocin-induced diabetes [44,45,66], chronic hypoglycemia [26,67–69], localized cerebral ischemia [29,70–72] and seizures [73,74]. In human brain samples, GLUT1 abundance has been reported to be decreased in the neocortex and hippocampus in Alzheimer's disease [75], and GLUT1 and GLUT3 have been reported to be decreased in the caudate and putamen in Huntington's disease [76]. Astrocytoma, a central nervous system tumor of glial cell origin, demonstrates an increase in GLUT1 and GLUT3 mRNA with increasing grade of malignancy; however, only immunoreactive GLUT3 protein is detected in the highest grade gliomas [77,78]. An absence of GLUT1 protein despite a relative high abundance of GLUT1 mRNA in high-grade gliomas has been explained by post-transcriptional modification of the GLUT1 mRNA: a 48-kDa cytosolic protein which is overexpressed in high-grade gliomas acts in trans to bind to specific sequences within the 3' untranslated region (3' UTR) of the GLUT1 mRNA. This interaction apparently results in destabilization of the GLUT1 transcript and gives rise to a discordance between the abundance of GLUT1 mRNA and immunoreactive protein [79].

Glucose transport and glucose transporter gene expression may be upregulated in situations of physiological stress to compensate for increased metabolic demand. Experimental brain ischemia produced by middle carotid artery occlusion results in upregulation of GLUT1 transcript both at the BBB and in brain parenchyma [70,71], a change which apparently is associated with a similar upregulation in GLUT1 protein [29]. The protective role of GLUT1 upregulation in brain has been observed in studies using replication-defective herpes simplex viral vectors to induce GLUT1 overexpression in neurons in vitro and in vivo: GLUT1 overexpression protects cultured neuronal cells against hypoxia-induced necrosis [80] and protects hippocampal neurons against ischemic injury and death in a rat model of stroke [81]. Compensatory increase of GLUT1 abundance on the BBB has been reported in a resected seizure focus of human brain [73], and upregulation of brain GLUT1 and GLUT3 have been reported in an animal model of chronic seizures [74]. The critical role of GLUT1-mediated BBB glucose transport in the provision of substrate to the brain is also evidenced by the report of seizures, developmental delay and hypoglycorrhachia (low cerebrospinal fluid glucose concentrations) in two infants with a haplosufficiency of GLUT1 [82].

Downregulation of glucose transport into brain has been reported in streptozotocin- and alloxan-induced as well as spontaneous diabetes in rats [44,45,66,83], a change that is secondary to a decrease in glucose transporter density at the BBB [45,84]. This decrease in BBB glucose transport and the consequent relative neuroglycopenia have been proposed as the basis for the neuroglycopenic symptoms experienced at normoglycemic levels in some individuals with poorly controlled diabetes when hyperglycemia is rapidly corrected [85–87]. The observation of decreased BBB glucose transport and/or GLUT1 expression in experimental diabetes, however, has not been universally accepted: studies from other laboratories have reported either unchanged or increased BBB glucose transport or GLUT1 density in this setting [69,88–90]. Furthermore, the concept of decreased cerebral metabolic rates due to a relative neuroglycopenia in individuals with poorly controlled diabetes has been called into question. Increased regional cerebral metabolic rates have been observed in streptozotocin diabetic rats following acute normalization of blood glucose levels [91], and more recently, positron emission tomography has failed to demonstrate a significant change in brain glucose metabolism or BBB glucose transport in individuals with poorly controlled diabetes, compared to non-diabetic controls [92]. Changes in BBB glucose transport and GLUT1 expression in the setting of suboptimally controlled diabetes is the subject of ongoing controversy [45,69,93].

With regard to changes in BBB GLUT1 in diabetes, an apparent discordance has been observed between the abundance of GLUT1 mRNA and protein in the BBB, similar to that seen in high-grade gliomas: in streptozotocin diabetic rats, BBB glucose transport and GLUT1 protein are downregulated [45], whereas the abundance of GLUT1 transcript is actually increased [94]. It is currently not known whether this apparent post-transcriptional modulation of BBB GLUT1 expression is also due to specific RNA-protein interactions such as those reported in high-grade gliomas [79].

**Brain glucose transport in iatrogenic hypoglycemia**

The hope inspired by the results of the Diabetes Control and Complications Trial (DCCT) [95] – that intensive insulin therapy with near-normalization of blood glucose levels leads to dramatic reductions in the risk of development and progression of microvascular complications – is tempered by the sobering realization that intensive insulin therapy is associated with a significant increase in risk for severe hypoglycemia [96]. Indeed, iatrogenic hypoglycemia has been referred to as the ‘principal obstacle in the treatment of insulin-dependent diabetes’ [97].

Accumulating clinical evidence has suggested that the increased risk of severe hypoglycemia experienced by individuals who undergo intensive insulin therapy is due...
to changes in the intricate feedback system which defends against declining blood glucose levels known as the counterregulatory response [97–102]. In a non-diabetic individual, falling blood glucose levels trigger a hierarchy of hormonal responses, which includes inhibition of insulin secretion, followed by the sequential release of glucagon, epinephrine and norepinephrine, cortisol, and growth hormone [103]. The net result of this crescendo of hormonal responses is to raise the blood glucose level. In non-diabetic individuals, the principal defense against hypoglycemia is production of glucagon from the α-cells of the pancreas and the resultant stimulation of hepatic gluconeogenesis [104]. In the setting of insulin-dependent diabetes, however, several defects exist in the counterregulatory response, including a loss of hypoglycemia-stimulated glucagon secretion relatively early in the course of the disease [105,106]. Consequently, individuals with Type 1 diabetes mellitus (DM) learn to depend on autonomic (predominantly adrenergic) 'warning symptoms', including palpitations, tremulousness, sweating, anxiety and warmth, to know when to seek carbohydrate replacement [104]. Normally, the counterregulatory response – and its associated autonomic warning symptoms – is triggered at a blood glucose concentration higher than that eliciting neuroglycopenic symptoms such as confusion, disorientation, stupor, coma and seizures [103]. The autonomic symptoms thus allow the individual with Type 1 DM to recognize the development of hypoglycemia and to seek corrective measures. Conversely, the loss of autonomic warning symptoms in the face of declining blood glucose levels that is associated with an absence or significant impairment of the counterregulatory response is responsible for the phenomenon of 'hypoglycemic unawareness' in which an individual with Type 1 DM develops cognitive dysfunction without prior warning symptoms. The practical consequence of this condition is the inability of an individual with diabetes to perceive and treat episodes of hypoglycemia before cognitive impairment and more severe hypoglycemia occur [107].

An accumulating body of evidence suggests that with the institution of intensive insulin therapy, the glycemic threshold for the counterregulatory response shifts to a lower blood glucose concentration [99,100,108]. Amiel and co-workers have used insulin clamp studies to demonstrate that the counterregulatory response in individuals with well-controlled diabetes, i.e. with HbA1c values of 7.6 ± 0.7%, is elicited at a lower blood glucose level than that seen in individuals with poorly controlled diabetes (HbA1c of 11.5 ± 1.7%) and in non-diabetic control subjects [99]. Furthermore, institution of intensive insulin therapy in a subset of the subjects with poor control is associated with the development of an identical impairment of counterregulation, like that seen in the well-controlled group [99]. The stimulus for the change in the counterregulatory threshold appears to be the often-unrecognized episodes of intermittent hypoglycemia which individuals with Type 1 DM under intensive insulin therapy experience on a daily basis [109,110].

Impairment of the counterregulatory response due to intermittent episodes of mild to moderate hypoglycemia may result in impaired perception of hypoglycemia, which in turn may act in a vicious cycle in predisposing an individual with Type 1 DM to further, more severe hypoglycemia [111]. Individuals in whom warning symptoms are lost, i.e. those with hypoglycemia unawareness, have an approximately 6-fold increased risk of severe hypoglycemia compared to individuals with Type 1 DM and an intact warning system [112], and although the cumulative effects of mild or moderate hypoglycemia on cognitive function are not certain, recurrent episodes of severe hypoglycemia are well known to result in permanent memory loss and cognitive problems [113].

As alluded to in the remarks above, the most vulnerable organ to the acute and chronic effects of hypoglycemia is the brain. The absolute reliance of the brain on glucose as its principal metabolic substrate and the relative lack of local stores of glycogen underlie the brain's selective vulnerability to hypoglycemia-mediated injury and its dependence on glucose transport across the BBB. Furthermore, under normal physiological conditions, phosphorylation of glucose within the neuronal cytosol by hexokinase occurs at a rate approximately half that of glucose transport and is normally the limiting step in brain glucose metabolism [1]. In hypoglycemia, however, brain hexokinase activity decreases out of proportion to glucose transport, and brain metabolism is limited by BBB glucose transport. Therefore, in hypoglycemia, GLUT1-mediated glucose transport at the BBB becomes the critical component in determining brain metabolism [22,114].

Experimental evidence has suggested a compensatory mechanism at the level of the BBB by which the brain protects itself against hypoglycemic injury. Using three different means of inducing sustained hypoglycemia in animals – repeated injections of long-acting insulin, and insulin infusion via subcutaneous osmotic pumps and via implanted insulinomas – McCall and colleagues demonstrated an upregulation of brain glucose extraction in comparison to normoglycemic control animals [67]. Koranyi and co-workers demonstrated an approximate 40% increase in total brain GLUT1 mRNA and protein in a rat model of sustained hypoglycemia [68]. Subsequent studies by Kumagai et al. of chronic hypoglycemia in rats confirmed McCall's results of increased BBB glucose transport and demonstrated that this increase was due to an increase in GLUT1 mRNA and protein specifically at the BBB [26]. The compensatory nature of hypoglycemia-induced changes in BBB glucose transport has been demonstrated in studies comparing the effects of acute versus chronic hypoglycemia on cerebral metabolic rate in rats (rCMRgluc) [50]. In contrast to the reductions seen in glucose transport and rCMRgluc in acute hypoglycemia, glucose transport is increased, and rCMRgluc and cortical function (as assessed by somatosensory evoked responses) are preserved in chronically hypoglycemic rats, compared to controls [50]. Taken together, these data suggest that a compensatory
upregulation of BBB glucose transport occurs in the setting of hypoglycemia, and that this increase preserves brain function in the face of low peripheral glucose levels.

The application of the findings described above to the understanding of the pathogenesis of the increased risk for severe hypoglycemia episodes during intensive insulin therapy must, however, take into account two considerations: the nature of the hypoglycemic stimulus, and the relationship between brain glucose transport and brain sensing of hypoglycemia. As mentioned previously, the type of hypoglycemia experienced by individuals under intensive insulin therapy is intermittent [109,110] and, therefore, changes in BBB glucose transport and GLUT1 expression reported in animal models of chronic or sustained hypoglycemia might not apply in the setting of intensive insulin therapy of Type 1 DM. Furthermore, it is unclear whether the changes in counterregulatory response to hypoglycemia are due to global increases in BBB glucose transport or to changes in transport in areas of the brain responsible for detection of declining blood glucose levels.

Boyle and colleagues recently investigated the relationship between changes in the counterregulatory response to hypoglycemia, hypoglycemia sensing and BBB glucose transport in individuals with various degrees of control of Type 1 DM and in non-diabetic subjects [46]. In a subset of individuals with the most intensively controlled diabetes (glycosylated Hb of 7.2±0.5%), impairment of counterregulation and hypoglycemia warning symptoms were associated with a preservation of BBB glucose transport. In contrast, individuals with less tightly controlled Type 1 DM and non-diabetic control subjects had a decrease in BBB glucose transport in response to the same hypoglycemic stimulus [46]. The results of this study suggest that the increased risk of severe hypoglycemia observed in intensively controlled Type 1 DM is due to a compensatory relative increase in total BBB glucose transport that results in a decreased counterregulatory response and blunted sensing of hypoglycemia by individuals under intensive insulin therapy. It is unclear, however, whether impairment of the counterregulatory response and hypoglycemia awareness observed in individuals under intensive insulin therapy are due to changes in whole brain glucose transport per se, i.e. whether preserved whole brain BBB glucose transport is responsible for the impairment in hypoglycemia sensing in these individuals. In the setting of intermittent hypoglycemia, the exact relationships between hypoglycemia sensing and counterregulatory and cognitive responses on the one hand, and regional or global changes in BBB glucose transport on the other, remain to be elucidated.

With regard to blood glucose sensing, it has long been hypothesized that the locus of glucose sensing responsible for the counterregulatory response to hypoglycemia is in the central nervous system. As demonstrated in canine studies by Biggers and co-workers, isolated hypoglycemia of the brain in the presence of systemic euglycemia may elicit a brisk counterregulatory response; in contrast, preservation of euglycemia in brain in the presence of systemic hypoglycemia abolishes the response [115]. In a series of elegant experiments in rats, Borg and co-workers provided evidence suggesting that the sensor is situated in the ventromedial hypothalamus (VMH): induction of localized, intracellular glycopenia in the VMH of rats by bilateral perfusion of 2-deoxyglucose into the two ventromedial hypothalamic nuclei results in a brisk counterregulatory response in the absence of hypoglycemia [116]. Conversely, perfusion of the VMH with concentrated glucose solutions abrogates the counterregulatory response in the setting of systemic hypoglycemia [117]. Other anatomical sites, including the liver [118,119], have been proposed as glucose sensing areas and may play a role in addition to, or in lieu of, the VMH glucose sensor; nonetheless, the studies cited above provide firm evidence that the primary sensor involved in counterregulation is localized in the hypothalamus, and in particular, the VMH [116,117].

The localization of different glucose transporter isoforms in the hypothalamus and whether changes occur in glucose transport and glucose transporter expression in the setting of intermittent hypoglycemia are unknown at present. In addition to neuronal GLUT3 and BBB GLUT1, the presence of the insulin-sensitive GLUT4 has been reported in the hypothalamus [51]. In a study investigating extra-pancreatic expression of glucokinase (the hexokinase isoform involved in glucose sensing in the β-cell), Jetton and co-workers expressed human growth hormone (hGH), acting as a reporter gene, in transgenic mice under the control of a glucokinase promoter [38]. Selective expression of hGH in the ventral hypothalamus in these studies suggested the possibility of glucokinase expression in this region of the brain, a finding supported by colocalization of immunoreactive glucokinase and the GLUT2 glucose transporter in the same region in normal mice [38]. The findings of Jetton's study are intriguing, because they suggest that the molecular machinery for glucose sensing in the β-cell, i.e. GLUT2-mediated glucose transport coupled with glucokinase activity, is recapitulated in an area of the brain which is responsible for hypoglycemia sensing. Additional observations, however, make the identification of the VMH as the ‘β-cell of the brain’ problematic: GLUT2 expression in the hypothalamus localizes to the arcuate nuclei, an area of the hypothalamus which is adjacent to the VMH [120,121]. GLUT2 and hexokinase, however, have not been reported in the VMH itself. Furthermore, the presence of the insulin-sensitive GLUT4 isoform in the hypothalamus raises the question of whether glucose transport in this area of the brain is modulated by insulin in addition to, or instead of, circulating blood glucose levels, a possibility suggested by 2-deoxyglucose brain autoradiographic studies in rats [122]. Clarification of possible changes in glucose transport in this critical region in the setting of intermittent hypoglycemia and the possible role of these changes in the increased risk of severe hypoglycemia experienced by individuals during intensive insulin therapy await further investigation.
Glucose transport and glucose transporters in retina

The neuroretina, which is among the most metabolically active tissues in the body [123], is nourished by transport of glucose across the endothelial cells of the capillaries of the inner BRB and from the choroidal vessels across the retinal pigment epithelium of the outer BRB [2]. Studies in rats have demonstrated a saturable transport process for glucose and other hexoses in the retina [124,125]; however, due to the presence of two barriers and due to uncertainties over the relative contribution of each barrier to the nourishment of the retina in man, the results of in vivo studies of glucose transport are difficult to interpret [126]. Studies in isolated bovine retinal capillaries have shown a sodium- and energy-independent, saturable transport process for hexoses, with a high affinity for the D-stereoisomer of glucose, followed by the analogs 2-deoxy-D-glucose and 3-O-methyl-D-glucose, as well as D-mannose, D-galactose and D-xylose [127]. In contrast, L-glucose, ribose and fructose are not transported to any appreciable extent in these preparations [127]. As in the endothelial cells of the brain, glucose uptake by retinal endothelia occurs in excess of metabolic rates (i.e. hexokinase activity), such that a large intracellular pool of free glucose is present within the endothelial cells [127]. In vivo measurements of retinal glucose transport and metabolism by NMR spectroscopy have recently confirmed these observations in the rabbit retina: glucose transport exceeds metabolic rates by approximately 16-fold [128]. A similar transport process for glucose has been described in isolated rat and bullfrog retinal pigment epithelium as well [129,130].

It is now known that the mammalian eye expresses GLUT1 in a variety of cell types [8,9,131,132]. In the human eye, GLUT1 is expressed in the retinal capillary endothelial cells, the retinal pigment epithelium, the non-pigmented epithelium of the ciliary body, the endothelium of the canal of Schlem, and the capillaries and posterior pigmented epithelium of the iris [8,131,133]. In the endothelial cells of the human and rat inner BRB, GLUT1 is expressed on both the luminal and abluminal membranes, a critical prerequisite for GLUT1's role in the transcellular transport of glucose to the neuroretina [134,135]. Furthermore, in the human inner BRB, a substantial portion (approximately 50%) of total cellular GLUT1 resides in cytosolic stores [135]. Because these transporters are not in contact with the plasma membrane surface, they are not available for glucose transport; however, in response to external stimuli, such as growth factors, these transporters may rapidly translocate to the plasma membrane where they may participate in mediating glucose entry into the endothelial cell.

In addition to the endothelial and epithelial cells of the inner and outer BRB, respectively, GLUT1 is expressed in cell bodies of the ganglion cell layer, the cell bodies of the photoreceptor elements and the Müller cells, a specialized glial cell which is oriented radially within the retina [131,133]. The finding of abundant expression of GLUT1 in Müller cells is in line with evidence of glucose transport and metabolism in isolated cell preparations [136] and supports the hypothesis that this glial cell serves as a nutrient-supporting cell of the retina [137,138]. In addition to GLUT1, Müller cells have been reported to express GLUT2, the liver and pancreatic β-cell glucose transporter, in their foot processes [139]. The selective function of this transporter in the Müller cells, if any, is currently not known. In the human retina, the expression of GLUT3 is restricted exclusively to the processes of neuronal cells of the inner synaptic layer [131], an observation which supports the role of GLUT3 as the major neuronal glucose transporter 12). Immunohistochemical studies have failed to document expression of the insulin-sensitive glucose transporter, GLUT4, and the fructose transporter, GLUT5, in the retina of the human eye [131].

Regulation of glucose transporter expression in retina

The factors mediating glucose transport and glucose transporter expression are not as well defined in the retina as they are in the brain. Expression of GLUT1 in the various cell types within the retina is preserved during development and is evident as early as 8 weeks of gestation [131]. In contrast, GLUT3 is not detected in the neuroretina in the fetus and localizes to the inner synaptic layer only in the adult retina [131]. As in other tissues, glucose transport and/or GLUT1 expression is modulated by hypoxia, growth factors and glucose in primary cultures of retinal endothelial cells, a cell culture model of the inner BRB [140,141] (A.K.K., in preparation). In cultured human retinal pigment epithelial cells, 2-deoxyglucose uptake and GLUT1 transcript have been shown to be upregulated in response to serum, insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) [142].

Inner blood retinal barrier glucose transport in retinopathy

Diabetic retinopathy is one of the principal microvascular complications of long-standing diabetes mellitus and represents the leading cause of blindness in working age adults in the USA [143,144]. Pathologically, the characteristic lesions and changes associated with diabetic retinopathy are localized to the retinal microvasculature of the inner BRB. These changes include loss of pericytes, increased tortuosity of retinal capillaries, formation of microaneurysms, leakage of plasma proteins, capillary obliteration, and, in the most severe stages of diabetic retinopathy, neovascularization [143]. The metabolic ‘culprit’ behind these changes appears to be chronic exposure of the retina to the hyperglycemia.
associated with long-standing diabetes, a proposition which rests on two major lines of clinical evidence: first, the severity of retinopathy is correlated with poor glycemic control [145], and conversely, as demonstrated by the landmark DCCT, near-normalization of blood glucose levels prevents or delays the progression of diabetic retinopathy in individuals with Type 1 DM [95].

The exact biochemical and molecular mechanisms that transduce the chronic hyperglycemia of diabetes into pathological changes in the retina have yet to be fully clarified. Attention has focused on the role of several pathways in this process, including oxidative stress, activation of protein kinase C (PKC), non-enzymatic glycation and direct toxic effects of glucose on endothelial function, replication and survival [146,147]. A common denominator, however, which connects hyperglycemia with these pathways is the exposure of the intracellular milieu of the retinal capillary endothelial cell to elevated blood glucose concentrations. Indeed, increased retinal endothelial cell glucose flux is assumed in the conceptualization of several of these pathways (Figure 1) [147,148].

As mentioned previously, glucose may gain entry into the endothelial cells of the inner BRB only via transport mediated by GLUT1 [2]. Furthermore, GLUT1-mediated transport operates at near-saturation levels at normal physiological blood glucose concentrations [6]. Therefore, elevations in blood glucose levels, such as those seen in diabetes mellitus, will have only a minor impact in increasing intracellular glucose concentrations. Because GLUT1 represents a unique portal of entry of glucose into the endothelial cells of the inner BRB, changes in retinal endothelial cell GLUT1 expression and glucose transport may have a major impact in providing substrate to the various pathogenic processes thought to underlie the development of diabetic retinopathy (Figure 1).

In a previous study, Kumagai and co-workers used immunogold electron microscopy to demonstrate localized upregulation of GLUT1 in retinal endothelial cells of postmortem retina specimens from three individuals with long-standing diabetes but without clinical evidence of diabetic retinopathy (Figure 2) [135]. On the luminal surface of the affected endothelia, there was a 20-fold increase in immunoreactive GLUT1, compared to microvessel profiles from non-diabetic retinas [135]. The upregulation of inner BRB GLUT1 was not uniform in the diabetic microvessels, but appeared to be localized, i.e. only certain capillaries exhibited increased GLUT1 abundance, while in other microvessels, GLUT1 abundance was not significantly different from that seen in non-diabetic specimens. The focal nature of the changes in GLUT1 expression in the diabetic inner BRB observed in this study is reminiscent of the focal histopathological changes and areas of inner BRB compromise which occur in the retinal microvasculature in diabetic retinopathy [143,149]. Upregulation of total retinal GLUT1 mRNA has been reported in galactosemic rats, which develop hyperhexosemia and retinal lesions similar to those seen in human diabetic retinopathy [150], and GLUT1 mRNA abundance has been reported to be increased in the retina of streptozotocin-induced diabetic rats, albeit to a level which was not statistically significant [151]. Taken together, these studies suggest that upregulation of retinal and, in particular, inner BRB GLUT1 occurs in the early stages of diabetic retinopathy, and that the resultant increase in inner BRB glucose transport plays a role in the progression of the pathological changes seen in later stages of the disease.

Figure 1. Proposed role of inner blood–retinal barrier GLUT1 in the development of microvascular pathology in diabetic retinopathy

Figure 2. GLUT1 immunogold electron microscopy of non-diabetic and diabetic inner blood–retinal barrier. (A) Non-diabetic retinal endothelial cell labeled with a 1:800 dilution of anti-GLUT1 antiserum, followed by 10 nm colloid gold-conjugated IgG. Sections were counterstained with uranyl acetate. GLUT1 immunogold is represented by discrete black dots along the luminal or abluminal membranes or scattered in the cytoplasm of the endothelial cell. Bar = 0.20 μm. (B) Representative endothelial cell from subpopulation of diabetic microvessels demonstrating dramatically increased GLUT1 expression. L, capillary lumen; N, nucleus of endothelial cell. Bar = 0.22 μm. Modified from Kumagai et al. [135]. Used with permission of Elsevier Science
In order to understand the role which changes in inner BRB GLUT1 expression may play in the pathogenesis of diabetic retinopathy, studies of the effects of different factors associated with the development of diabetic retinopathy on inner BRB glucose transport and GLUT1 expression are needed. In a recent publication, Takagi and colleagues demonstrated upregulation of GLUT1 transcript and protein in primary cultures of bovine retinal endothelial cells exposed to hypoxia [140]. The hypoxic conditions (0.5% O₂) increased GLUT1 mRNA by approximately 9-fold and GLUT1 protein and 2-deoxyglucose transport by 2.5- and 3-fold, respectively, after 12 h. These changes were partially mimicked by A2 adenosine receptor agonists and partially inhibited by A2 receptor antagonists. Furthermore, an inhibitor of protein kinase A (PKA) partially abolished the hypoxia-mediated increase in GLUT1 mRNA abundance. These experiments demonstrate that hypoxia can upregulate GLUT1 expression and glucose transport in vitro, and that this process occurs through an adenosine-A2 receptor-PKA pathway [140]. While hypoxia – a factor associated with advanced non-proliferative diabetic retinopathy – provides a potent stimulus for increases in GLUT1 expression in retinal endothelial cells [140], growth factors or other metabolic perturbations (e.g. oxidative stress, increased PKC activity) present early in the development of diabetic retinopathy may play a role in modulating inner BRB GLUT1 expression. Studies from the author’s laboratory have demonstrated an enhancement of glucose transport in primary bovine retinal endothelial cell cultures by vascular endothelial growth factor (VEGF) (A.K.K., in preparation). VEGF, a cytokine that selectively acts on endothelial cells to increase vascular permeability and stimulate angiogenesis [152], has been demonstrated to play a major role in the development of proliferative retinopathy [153–155]. Although there is some controversy [156], numerous studies in humans [157,158] and animal models [159,160] suggest that VEGF may play a role in the non-proliferative stage of the disease as well.

With regard to the direct effects of glucose on retinal vascular cell glucose transport, Mandarino and colleagues have reported that exposure to elevated glucose concentrations for 5 days downregulates both glucose transport and GLUT1 mRNA and protein in primary cultures of retinal pericytes, but has no effect on glucose transport retinal endothelial cells [141]. Changes in endothelial expression of GLUT1 transcript or protein were not investigated in this study. Knott and co-workers have reported upregulation of GLUT1 and GLUT3 mRNA in response to elevated glucose concentrations in human retinal endothelial cell cultures [161]. The observations of Knott and co-workers with respect to glucose-mediated changes in cultured retinal endothelial cell GLUT3 expression, however, are difficult to interpret, since immunoreactive GLUT3 is not detected in plasma membranes of cultured retinal endothelial cells [141], and more crucially, GLUT3 is not present in the retinal endothelial cells of the inner BRB in vivo [131].

A possible role of upregulated GLUT1 expression in the pathogenesis of diabetic complications is not unique for the retina. In a cell culture model of renal mesangial cells, another cell type which is selectively vulnerable to the effects of long-standing hyperglycemia, Heilig and co-workers induced mesangial cell GLUT1 overexpression via retroviral-mediated transfection. The investigators observed increased intracellular concentrations of sorbitol and myo-inositol and accumulation of extracellular matrix components in the GLUT1-overexpressing cultures grown in normal glucose when compared to lacZ transfected controls [162]. In other words, in mesangial cells in culture, overexpression of GLUT1 recapitulates at least some of the phenotypic characteristics of the diabetic milieu. While mesangial cells in culture differ from cultured retinal endothelium in that mesangial cell GLUT1 is positively regulated by glucose [163], factors associated with the diabetic milieu in the retina, such as localized hypoxia and growth factor expression, may serve as the initial impetus for GLUT1 upregulation and thus expose the endothelial cells of the retina to elevated intracellular glucose concentrations. In this manner, localized upregulation of inner BRB GLUT1 expression may exacerbate the deleterious effects of hyperglycemia on the retinal microvasculature and play a critical role in the development and progression of diabetic retinopathy.

Conclusions

Facilitated transport of glucose across the continuous endothelium comprising the BBB and inner BRB ensures continual delivery of this essential metabolite into the brain and retina, respectively. As described above, this transport process, and the glucose transporter that mediates it, may be modulated by a variety of factors, including hypoxia, growth factors, physiologic stress and glucose itself. Changes in endothelial glucose transport and in the abundance of GLUT1 on the endothelial plasma membrane in these barriers may have profound consequences in glucose delivery to neural tissue and may be intimately connected with the development of complications associated with diabetes mellitus and its treatment. In the brain, hypoglycemia-associated compensatory upregulation of BBB glucose transport may preserve BBB glucose transport and brain glucose metabolism in the face of low peripheral blood glucose concentrations. The ultimate consequence of this increase, however, may be deleterious: changes in brain glucose transport in the setting of intensive insulin therapy may result in a blunting of the counterregulatory response to hypoglycemia, a decreased awareness of autonomic warning symptoms, and a substantially increased risk of further, more severe hypoglycemic episodes. In the retina, increased abundance of GLUT1 on the plasma membranes of retinal capillary endothelial cells resulting from exposure to various factors associated with the development of diabetic retinopathy (e.g. hypoxia, growth factors) may lead to increased glucose delivery to neural tissue, thus exacerbating the pathogenesis of diabetic retinopathy.

transport into the endothelial cells (Figure 1). This increased influx, and the subsequent increase in intracellular glucose concentrations, may exacerbate the effects of hyperglycemia on the endothelial cell, in effect by ‘adding fuel to the fire’ of the various biochemical and molecular processes leading to the development of the characteristic structural and functional changes in the inner BRB associated with diabetic retinopathy. The studies described above suggest the critical importance of BBB glucose transport to changes in brain glucose sensing and to the increased risk of severe hypoglycemia experienced by individuals attempting to achieve optimal glycemic control. With regards to diabetic retinopathy, a paradigm is proposed in which changes in glucose transport in the endothelia of the inner BRB participate in the development of the microvascular lesions in the retina in long-standing diabetes mellitus. Clarification of the exact role of changes in BBB and inner BRB glucose transport and glucose transporter expression, and the molecular processes underlying such changes, in these complications of diabetes are the subject of ongoing investigation.

Acknowledgements

The author would like to thank Drs Hirohito Sone and Chardpra-Orn Ngarmukos and Ms Baljeet Deo for their efforts in many of these studies, and Drs Frank C. Brosius III, Rubén Boado, Christin Carter-Su, Douglas A. Greene and William M. Pardridge for many valuable discussions. This work was supported by National Institutes of Health grant EY000369 and grants from the Juvenile Diabetes Foundation and the American Diabetes Association. This work was also supported in part by National Institutes of Health grant 5PO6DK-20572, which supports the Michigan Diabetes Research and Training Center.

References


74. Gronlund KM, Gerhart DZ, Leino RL, McCall AL, Drewes IR.


Glucose Transporters in Brain and Retina


