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Biochimica et Biophysica Acta 1193 (1994) 24–30

BBA Biochimica et Biophysica Acta

Differential glycosylation of the GLUT1 glucose transporter in brain capillaries and choroid plexus

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Received 23 December 1993; revised manuscript received 24 March 1994

Abstract

The sodium-independent GLUT1 glucose transporter is expressed in high density in human erythrocytes and in tissues which serve a barrier function. In the polarized endothelial cells of the brain capillaries, which comprise the blood-brain barrier (BBB), GLUT1 is expressed on both apical and basolateral membranes; however, in the epithelium of the choroid plexus, GLUT1 expression is restricted to the basolateral surface. The present study examined whether these differences in subcellular localization of GLUT1 at the BBB and choroid plexus could be correlated with differential N-linked or O-linked glycosylation of the protein. Western blot analysis of solubilized brain capillaries (BC) and choroid plexus (CP) revealed that while the BC GLUT1 had an average molecular mass identical to that of the purified human erythrocyte transporter (54 kDa), the CP GLUT1 was of lower molecular mass (47 kDa). Treatment of brain capillaries and choroid plexus with *N*-glycanase resulted in a shift in the mobility of the GLUT1 of both samples to a lower molecular mass of 42 kDa; however, in contrast, treatment with *O*-glycanase produced no change in the mobility patterns of GLUT1, but did result in O-linked deglycosylation of another BBB marker, γ-glutamyl transpeptidase. In conclusion, BBB and choroid plexus GLUT1 are subject to differential N-linked glycosylation with the protein having an N-linked carbohydrate side chain of higher molecular mass at the BBB in comparison to the choroid plexus.

Key words: Glucose transporter; Blood-brain barrier; Choroid plexus; Glycosylation; Subcellular localization; (GLUT1)

1. Introduction

GLUT1 is a 492-amino acid protein which is one of multiple isoforms of the sodium-independent glucose transporter gene family [4,27]. As predicted by its primary amino acid sequence, the GLUT1 protein is arranged in twelve membrane-spanning domains [27] and has a single glycosylation site, the asparagine residue at position 45 (Asn^{45}) of the first extracellular loop [27]. GLUT1 is expressed on the cell surfaces of a wide variety of tissues, including those which serve a barrier function [20,32]. In the brain, GLUT1 has recently been identified as the principal transporter isoform mediating delivery of glucose across the endothelial cells of the capillaries which form the blood-brain barrier [28] and has been demonstrated on the epithelium of the choroid plexus [13,19,21,32]. Although GLUT1 is detected in different endothelial and epithelial barriers, immunoreactive GLUT1 protein is not evenly distributed on the cell surfaces of the tissues in which it is expressed. On brain capillary endothelial cells GLUT1 is asymmetrically distributed on apical and basolateral surfaces [12], whereas expression of GLUT1 is restricted to the basolateral surfaces of the epithelium of the choroid plexus [13,19,20]. Conversely, GLUT1 is distributed to the apical surface of ependymal epithelium lining a region of the third ventricle in brain [13].

Abbreviations: BBB, blood-brain barrier; BC, brain capillary; BCM, brain cell membrane; CP, choroid plexus; GGTP, γ-glutamyl transpeptidase; anti-GLUT1, antiserum directed against the C-terminus of GLUT1 protein; anti-GGTP, antiserum containing a mixture of antisera directed against the heavy and light chains of GGTP.

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SSDI 0005-2736(94)00098-A

The differential subcellular localization of GLUT1 to apical vs. basolateral surfaces of cells comprising tissue barriers may be due to differential post-translational modification of the transporter protein. On sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), GLUT1 in human erythrocytes and bovine brain capillaries exhibits comparable mobility, with a broad band of average molecular mass of 52 kDa [28]. The large range in GLUT1 molecular mass has been attributed to heterogeneous glycosylation of the transporter protein [3,18]. In contrast to the GLUT1 found on bovine brain capillaries, the GLUT1 in capillary-depleted bovine brain cell membranes (BCM) is relatively homologous, characterized by a single band with a molecular mass of 42 kDa [28]. The difference in molecular mass of GLUT1 in brain capillaries and brain cell membranes may be due to differences in posttranslational modifications of the GLUT1 protein, e.g., differences in the complexity of carbohydrate side chains attached to GLUT1 at Asn^{45} [28].

In the present study, we employed Western analysis of GLUT1 of isolated bovine and rat brain capillaries and choroid plexus to investigate whether the differences in subcellular localization of GLUT1 on brain capillary endothelial cells and choroid plexus epithelium could be correlated with differences in molecular mass. In addition, we performed deglycosylation experiments to investigate the possible role which N-linked or O-linked glycosylation may play in the posttranslational modification of the GLUT1 protein in these two barrier systems.

2. Experimental

2.1. Materials

Fresh bovine brains were obtained from a local slaughterhouse. Male Sprague-Dawley rats were purchased from Harlan-Sprague-Dawley (Indianapolis, IN, U.S.A.). Deoxycytidine 5′-triphosphate tetra(triethylammonium) salt [α-^{32}P], 5000 Ci/mmol and Genescreen Plus membranes were purchased from DuPont-NEN (Boston, MA, USA). The ABC (Elite) kit for Western blot analysis was from Vector Laboratories (Burlingame, CA, USA). Recombinant *N*-glycanase (*N*-glycosidase F), neuraminidase, and *O*-glycanase (endo-α-*N*-acetylgalactosaminidase) were purchased from Genzyme Corporation (Boston, MA, USA). Partially purified bovine kidney γ-glutamyl transpeptidase and all other reagents were purchased from Sigma (St. Louis, MO, USA). The bovine BBB glucose transporter cDNA has been described previously [6]. The mouse actin cDNA was generously provided by Michael J. Getz, Ph.D. (Mayo Foundation, Rochester, MN, USA).

2.2. Brain capillary, choroid plexus and erythrocyte glucose transporter isolation

All procedures involving animals were in accordance with the requirements of the institutional animal research review committee. Bovine and rat brain capillaries were isolated as described previously [15,29]. Choroid plexus was isolated from male Sprague-Dawley rats after deep halothane anesthesia and decapitation and from bovine brains. After thorough washing to remove excess blood, choroid plexus protein was solubilized in 15 mM Tris, 2% (w/v) sodium dodecyl sulfate (SDS) and 0.1 mM ethylenediaminetetraacetic acid (EDTA) according to methods previously described for solubilization of brain capillaries [28]. The isolation of capillary-depleted rat brain synaptosomal membranes (also called brain cell membranes) has been described previously [28]. Human erythrocyte glucose transporter was purified with the method of Baldwin and Lienhard [3] as described previously [28].

2.3. Poly(A)$^+$ RNA isolation

Poly(A)$^+$ RNA was isolated from bovine brain capillaries or choroid plexus using a one-step procedure described previously [8].

2.4. Western blot analysis

Aliquots of 10 μg of solubilized rat brain capillaries or brain cell membranes and 20 μg of solubilized rat choroid plexus were applied to 12% SDS polyacrylamide gels, and electrophoresis was performed as described previously [28] except that a minigel format was used. Samples were not heated prior to SDS-PAGE in order to avoid aggregation of the GLUT1 protein [3]. Following electrophoresis, the isolated proteins were transferred to Genescreen Plus membranes and Western immunoblot analysis was performed using antiserum directed against the carboxyl terminus of GLUT1 (see below). As a control, identical Western blots were probed using the same antiserum after preabsorption with a molar excess of the carboxyl terminus synthetic peptide. The GLUT1 protein was identified using an avidin-biotin-peroxidase method (ABC Elite) as described previously [28].

In another series of experiments, Western blotting was performed as described above on triplicate 20-μg aliquots of solubilized bovine brain capillaries and bovine choroid plexus, and the relative amounts of GLUT1 detected were quantified by a Microscan 1000 two-dimensional video gel analyzer (Technology Resources, Nashville, TN, USA) as described previously [11].

2.5. Deglycosylation experiments

N-Deglycosylation. N-Linked carbohydrate groups were cleaved from the GLUT1 protein in brain capillaries and choroid plexus using recombinant *N*-glycanase (*N*-glycosidase F). Aliquots of either 20 μg of SDS-solubilized bovine or rat capillaries or 40 μg of rat or bovine choroid plexus were added to a 54-μl volume of 15 mM sodium phospate buffer (pH 7.4) containing 1% (v/v) Nonidet NP-40, 2.0 TIU/ml aprotinin, 10 mM phenylmethylsulfonyl fluoride (PMSF) and 6 units/ml *N*-glycanase, with a final SDS concentration of 0.1%. As a control, 10% (v/v) glycerol was substituted for *N*-glycanase in otherwise identical reaction mixtures. After overnight incubation at room temperature, the reaction was stopped with the addition of 4 × SDS sample buffer. The samples, along with an untreated 10-μg aliquot of solubilized rat brain capillaries, were applied to 12% polyacrylamide gels, and SDS-PAGE and Western blotting were performed as described above. Owing to high nonspecific staining in bovine, as compared to rat, choroid plexus, most studies were performed with rat choroid plexus after initial studies showed that the immunoreactive GLUT1 migrated identically in the two species. With regards to detection of GLUT1 in brain capillaries, initial studies showed that GLUT1 was identical in bovine and rat specimens. Therefore, the majority of studies were performed with bovine brain capillaries owing to the greater supply of bovine brain tissue.

O-Deglycosylation. The presence of O-linked carbohydrate groups on bovine brain capillary GLUT1 protein was investigated using neuraminidase and *O*-glycanase (endo-α-*N*-acetylgalactosaminidase). Aliquots of 20 μg of solubilized bovine brain capillaries were incubated in the presence of 1.2 units/ml neuraminidase at room temperature for 30 min. Nonidet NP-40, aprotinin and PMSF were added to the reaction mixtures as described above. The samples were subsequently treated with 24 units/ml *O*-glycanase at room temperature for 8 h. For comparison, identical samples were treated in the same manner with substitution of 10% (v/v) glycerol for neuraminidase and *O*-glycanase. As a positive control, 5-μg aliquots of partially purified bovine kidney γ-glutamyl transpeptidase (GGTP), which possesses O-linked carbohydrate groups [5], were treated with and without neuraminidase and *O*-glycanase. The reaction was stopped with the addition of 4 × SDS sample buffer, and Western blotting was performed as described above with the following modification: after transfer of the proteins to Genescreen membranes, the lanes containing the GGTP were cut away and probed with anti-GGTP antiserum, which consisted of a mixture of antisera directed against synthetic peptides corresponding to the heavy and light chains of bovine GGTP (see below). As a control, identical Western blots of GGTP were probed with anti-GGTP antiserum which had been preabsorbed with the corresponding synthetic peptides.

2.6. Antisera

Three different rabbit polyclonal antisera prepared against synthetic peptides were used. The anti-GLUT1 antiserum was directed against a synthetic peptide corresponding to amino acids 480–492 of the carboxyl terminus of GLUT1 [10,22,27], and has been described previously [28]. Since the different glucose transporter isoforms do not share amino acid sequence homology at the carboxyl terminus [4], production of anti-GLUT1 antiserum directed against the C-terminus of the protein confers specificity of the antiserum to the GLUT1 isoform. The anti-GGTP antisera consisted of a 1:1 mixture of one antiserum directed against a 21-amino acid synthetic peptide corresponding to amino acids 359–379 at the carboxyl terminus of the heavy chain of rat GGTP [26] and a second antiserum directed against an 18-amino acid synthetic peptide corresponding to amino acids 551–568 at the carboxyl terminus of the light chain of rat GGTP [26]. The antisera were raised in rabbits, and the rat GGTP heavy chain antiserum has been described previously [9]. All synthetic peptides were administered as immunogens to rabbits as glutaraldehyde cross-linked conjugates with bovine thyroglobulin. The specificity of the anti-light chain antiserum was demonstrated by Western blot analysis, which showed that the antiserum specifically illuminated the 22 kDa light chain of bovine GGTP, and by light microscopic immunocytochemistry of 6-μm sections of formalin fixed, paraffin embedded sections of rat kidney, which showed illumination of the brush border of renal tubular epithelium. Both the Western blot and immunocytochemical signals were eliminated by absorption of the anti-light chain antiserum by the corresponding synthetic peptide (data not shown).

2.7. Northern blot analysis

Bovine brain capillary or choroid plexus poly(A)$^+$ RNA (5 μg and 1 μg, respectively) was applied to 1.1% agarose-2.2 M formaldehyde gels, followed by blotting onto GeneScreen Plus membranes and hybridization with [^{32}P]GLUT1 or [^{32}P]actin cDNAs, as described previously [7]. After autoradiography using intensifying screens, Northern blots were quantified by scanning video densitometry of the X-ray film, as described previously [11]. All samples were analyzed in triplicate.

3. Results

The Western blot analysis of rat brain capillaries (rBC), rat choroid plexus (rCP), capillary-depleted rat

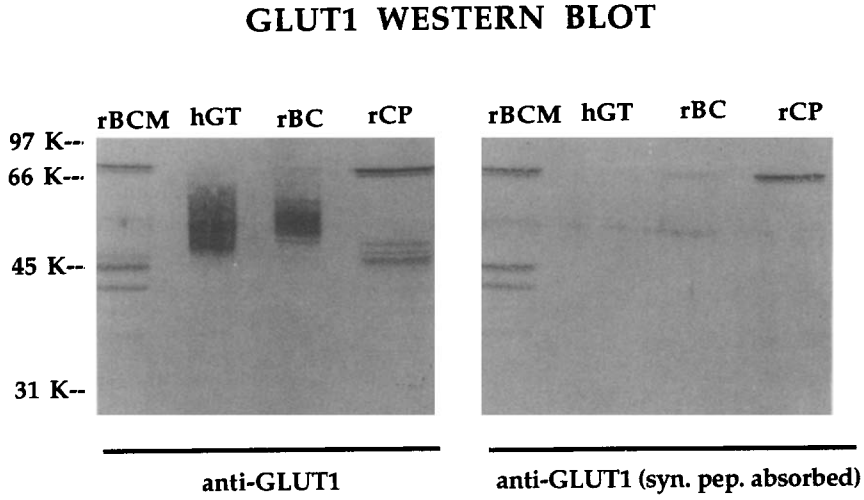

Fig. 1. Western blot comparing immunoreactive GLUT1 expression in purified human erythrocyte glucose transporter, rat brain capillaries, rat choroid plexus and rat brain cell membranes. Aliquots of either 10 μg of solubilized rat brain capillaries (rBC) and rat brain cell membranes (rBCM), 20 μg of solubilized rat choroid plexus (rCP) or 10 ng of purified human erythrocyte glucose transporter (hGT) were subjected to SDS-PAGE. Separated proteins were probed with a 1:500 dilution of either a polyclonal antiserum directed against a synthetic peptide corresponding to the carboxyl terminus of GLUT1 (anti-GLUT1), left panel, or identical antiserum which had been preabsorbed with the carboxyl terminus synthetic peptide (anti-GLUT1, syn. pep. absorbed), right panel. The positions of the molecular mass markers are indicated.

brain cell membranes (rBCM) and purified human erythrocyte glucose transporter (hGT) probed with the anti-CT GLUT1 antiserum is shown in Fig. 1. The GLUT1 of rat brain capillaries and human erythrocytes eluted as a broad band with an average molecular mass of 52 kDa. The immunoreactive GLUT1 protein of the capillary-depleted rat brain cell membrane was characterized by a single band of lower molecular mass of 46 kDa. The rat choroid plexus GLUT1 was characterized on Western blot by a triplet of immunoreactive proteins migrating at 46–48 kDa. Preabsorption of the anti-GLUT1 antiserum with synthetic peptide resulted in the loss of immunoreactivity of the GLUT1 of the brain capillaries, brain cell membranes and choroid plexus; preabsorption did not, however, alter nonspecific binding of the antiserum to proteins of approximate molecular mass of 70 kDa in all three tissues and of 45 kDa and 40 kDa in the brain cell membranes (Fig. 1).

Fig. 2 demonstrates the results of treatment of solubilized brain capillaries and choroid plexus with N-glycanase. The immunoreactive GLUT1 detected in the untreated rat brain capillaries (lane 1) exhibited an identical mobility on SDS-PAGE as that for the un-

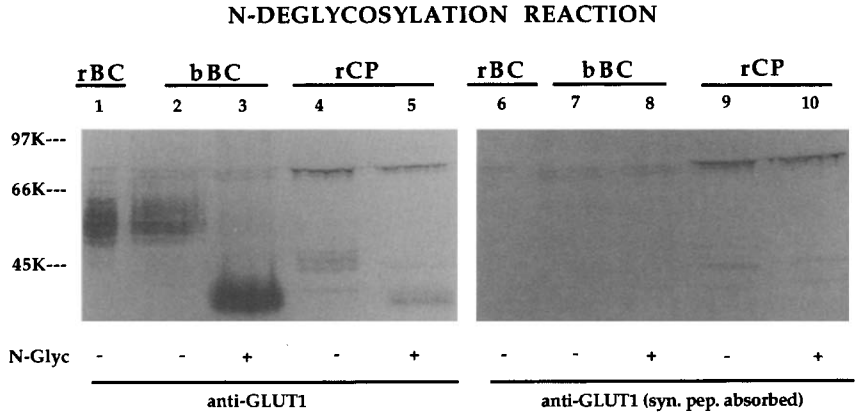

Fig. 2. Western blot analysis of N-deglycosylation reaction. Aliquots of 20 μg of solubilized bovine brain capillaries (bBC, lanes 2, 3, 7 and 8) or 40 μg of solubilized rat choroid plexus (rCP, lanes 4, 5, 9 and 10) were treated with (+) or without (−) 6 U/ml of *N*-glycosidase F (*N*-glyc.) overnight at RT, as described in the Experimental section, and subjected to Western blotting with a 1:500 dilution of either anti-C terminal GLUT1 antiserum (anti-GLUT1) or with identical antiserum preabsorbed with synthetic peptide corresponding to the C terminus of GLUT1 (anti-GLUT1, syn. pep. absorbed). An untreated 10-μg aliquot of rat brain capillaries (rBC) was applied for comparison (lanes 1 and 6). Western blotting was performed as described in Fig. 1 and in Experimental. Relative molecular masses corresponding to the migration of biotinylated molecular mass standards are shown on the right.

treated bovine sample (lane 2), with an average molecular mass of 52 kDa. N-deglycosylation of bovine brain capillaries resulted in a shift of the band corresponding to GLUT1 to a lower mean molecular mass of approximately 42 kDa (lane 3). In a similar manner, N-deglycosylation of rat choroid plexus resulted in a shift of choroid plexus GLUT1 from an average molecular mass of 47 kDa (lane 4) to approximately 42 kDa (lane 5). The average molecular masses of the bovine brain capillary and rat choroid plexus GLUT1 after N-deglycosylation were identical (42 kDa). A separate series of experiments demonstrated identical changes in the mobility of GLUT1 after N-deglycosylation between rat and bovine brain capillaries (data not shown). Furthermore, treatment of solubilized bovine choroid plexus with *N*-glycanase resulted in an identical shift of immunoreactive GLUT1 as that for rat choroid plexus (data not shown). However, the specific staining of immunoreactive GLUT1 was weak relative to the staining of nonspecific bands in the bovine choroid plexus. Therefore, the majority of experiments employed rat choroid plexus to examine the shift in mobility with N-deglycosylation (Fig. 2, lane 5).

O-Deglycosylation of solubilized bovine brain capillaries failed to produce a shift of immunoreactive GLUT1 to a lower molecular mass (Fig. 3). In contrast, both the 90 kDa and 25 kDa bands corresponding to the heavy and light chains, respectively, of bovine GGTP demonstrated a shift to lower molecular mass after treatment with neuraminidase and *O*-glycanase (Fig. 3).

Differential processing of the primary transcript encoding for the GLUT1 protein would result in mRNAs of different size in brain capillaries versus choroid plexus. This possibility was examined by isolation of poly(A)$^+$ RNA from bovine brain capillaries (bBC) and bovine choroid plexus (bCP) and Northern blot analysis. However, the GLUT1 transcripts of the brain capillaries and choroid plexus were identical in size, 2.9 kb (Fig. 4).

Results of scanning video densitometry of Western blots of triplicate samples of solubilized bovine brain capillaries and choroid plexus probed for GLUT1 are shown in Table 1. When normalized for differences in amounts of protein, the relative amount of immunoreactive GLUT1 detected in bovine brain capillaries was approximately 13 times that in choroid plexus. The ratio of GLUT1 mRNA/actin mRNA in brain capillaries relative to choroid plexus was 30 to 1 (Table 1).

4. Discussion

The findings of the present study are consistent with the following conclusions. First, the GLUT1 expressed in brain capillaries comprising the BBB has a different molecular size and mobility on SDS-PAGE as the immunoreactive GLUT1 in either choroid plexus or in

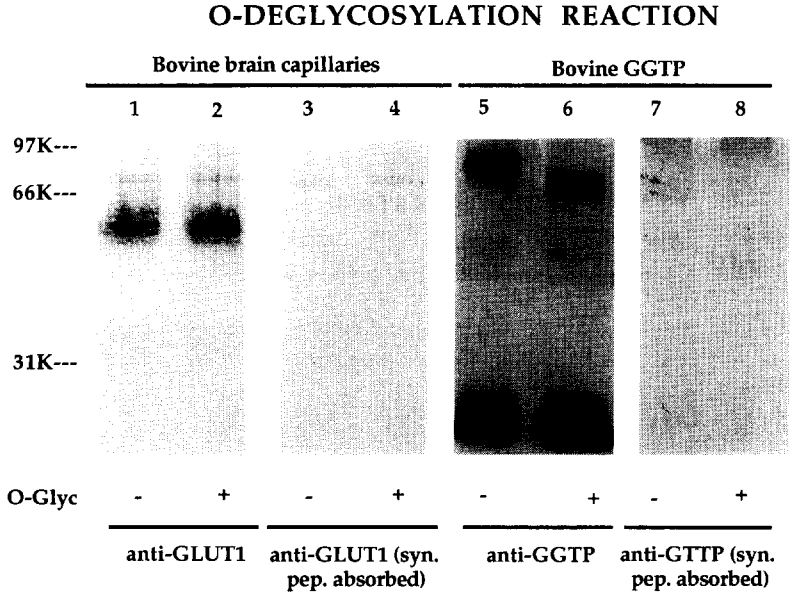

Fig. 3. Western blot analysis of O-deglycosylation reaction. Aliquots of 20 μg of solubilized bovine brain capillaries (lanes 1–4) were treated with (+) or without (−) 1.2 U/ml neuraminidase for 30 min. at RT, followed by 24 U/ml of endo-α-*N*-acetylgalactosaminidase (*O*-glyc) for 8 h at RT, as described in Experimental. As a positive control, 5-μg aliquots of partially purified bovine kidney γ-glutamyl transpeptidase (GGTP, lanes 5–8) were treated simultaneously in an identical manner. Western blotting was performed with a 1:500 dilution of either anti-C terminal GLUT1 antiserum (lanes 1 and 2) or a mixture (1:1) of polyclonal antisera directed against synthetic peptides corresponding to the heavy chain or light chain of GGTP (anti-GGTP, lanes 5 and 6). Nonspecific antisera binding was assessed by treatment of identical Western blots with anti-GLUT1 (lanes 3 and 4) or anti-GGTP (lanes 7 and 8) antisera which had been preabsorbed with the corresponding synthetic peptides.

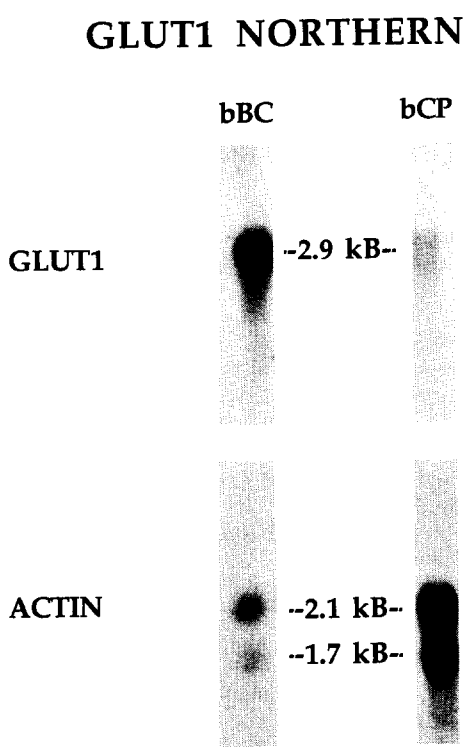

Fig. 4. Northern blot analysis of poly(A)$^+$ RNA from bovine brain capillaries and choroid plexus. Aliquots of 1 μg of bovine brain capillary (bBC) or 5 μg of bovine choroid plexus (bCP) poly(A)$^+$ RNA were applied to 1.1% agarose/2.2 M formaldehyde gels and subjected to electrophoresis. A 5-μl aliquot of a standard RNA ladder was applied for comparison. Transferred poly(A)$^+$ RNA was then sequentially probed with ^{32}P-labeled GLUT1 or actin cDNA, as described previously [9]. The GLUT1 transcripts of the bBC and bCP (upper panels) were identical in size and were represented by bands of 2.9 kb in length. Messenger RNA for actin (lower panels) were characterized in both bBC and bCP by 2 transcripts of 2.1 kb and 1.7 kb representing the β/γ-actin and α-actin mRNAs, respectively [6].

Table 1
GLUT1 mRNA and protein in bovine brain capillaries and bovine choroid plexus

Parameter	BC	CP	BC/CP
IR GLUT1 (A/mg$_p$)	6.3 ±0.1	0.50 ±0.01	13 :1
GLUT1 mRNA	0.48±0.12 [a]	0.062±0.015 [b]	39 :1 [c]
Actin mRNA	0.16±0.10 [a]	0.43 ±0.13 [b]	1.9:1 [c]
GLUT1/actin mRNA	4.8 ±1.4	0.16 ±0.04	30 :1

Western blotting of 20-μg aliquots of solubilized bovine brain capillaries (BC) and choroid plexus (CP) was performed with the antiserum directed against the C-terminus of GLUT1. Immunoreactive GLUT1 (IR GLUT1) was quantified by scanning video densitometry and expressed in arbitrary absorption units per milligram protein (A/mg$_p$). Northern blotting analysis was performed on 1-μg aliquots of BC or 5-μg aliquots of CP poly(A)$^+$ RNA with ^{32}P-labeled cDNAs for either GLUT1 or actin. Quantification of GLUT1 and actin mRNA was performed by scanning video analysis of the X-ray film and expressed in arbitrary absorption units per 1- or 5-μg of poly(A)$^+$ RNA, as is indicated in the footnote. All samples were run and analyzed in triplicate.

[a] Absorption units per 1 μg poly(A)$^+$ RNA.

[b] Absorption units per 5 μg poly(A)$^+$ RNA.

[c] Absorption units normalized to 1 μg poly(A)$^+$ RNA.

capillary depleted brain cell membranes (Fig. 1); this difference is unlikely due to alterations in the length of the polypeptide chain, since the mRNA from both brain capillaries and choroid plexus is the same size (Fig. 4). Second, the differences in molecular mass between BBB and choroid plexus GLUT1 arise from differential N-linked glycosylation (Fig. 2), while no evidence is obtained for O-linked glycosylation of GLUT1 (Fig. 3).

Comparison of the immunoreactive GLUT1 of brain capillaries, brain cell membranes and choroid plexus in Fig. 1 demonstrates that the mobility on SDS-PAGE of immunoreactive GLUT1 from choroid plexus at 46–48 kDa is intermediate between that of brain capillaries (52 kDa) and brain cell membranes (46 kDa). There thus appears to be a spectrum of sizes of GLUT1 in different brain compartments with brain capillary > choroid plexus > brain cell membrane (Fig. 1).

The present study provides evidence that the differential mobility on SDS-PAGE of immunoreactive GLUT1 isolated from brain capillaries and choroid plexus arises from differences in the complexity of N-linked carbohydrate groups. Following N-deglycosylation, the mobility of immunoreactive GLUT1 at either the BBB or the choroid plexus is identical (Fig. 2). Previous studies have demonstrated similar findings in comparing changes in mobility of GLUT1 following N-deglycosylation of bovine brain capillaries and capillary-depleted brain cell membranes [28]. The presence of a broad band on Western blot corresponding to GLUT1 in bovine brain capillaries or rat choroid plexus following N-deglycosylation in the present study (Fig. 2) suggests either incomplete deglycosylation or the presence of multiple GLUT1 proteins. The latter possibility, however, is unlikely given the identical size of the GLUT1 transcript in both the BBB and choroid plexus (Fig. 4). There still remains the possibility that GLUT1 from either the BBB or choroid plexus is subject to O-linked glycosylation. Studies in other systems have shown that the differential cellular routing of membrane proteins is regulated in part by O-linked carbohydrate attached to serine/threonine residues [30]. Although consensus amino acid sequences specifying N-glycosylation are well-known [27], comparable consensus sequences for O-linked glycosylation have not yet been determined [24]. Therefore, we examined the hypothesis that GLUT1 may be subject to O-linked glycosylation. However, the present studies do not provide evidence for O-linked glycosylation of GLUT1, although the methods used in these studies were capable of detecting O-linked glycosylation of GGTP (Fig. 3). This enzyme, like GLUT1, is a BBB membrane marker [16], and is subject to O-linked glycosylation on both heavy and light chains [5].

The heterogeneity of GLUT1 glycosylation in different barriers in brain, such as the BBB or choroid

plexus, is believed to have a functional role that may participate in regulating glucose transporter kinetic activity or differential subcellular localization of the glucose transporter [1,2,14]. Previous studies have shown that immunoreactive GLUT1 is distributed to both the lumenal and ablumenal membranes of the brain capillary endothelium [12]. In epithelial barriers, the transporter is usually distributed to the basolateral membrane [17,25,33], and immunoreactive GLUT1 is selectively localized to the basolateral membrane of the choroid plexus epithelium [12,19,20]. These morphologic findings in conjunction with the present biochemical results suggest that the subcellular targeting of GLUT1 to the apical (lumenal) versus the basolateral (ablumenal) membrane of barrier cells in brain may in part be due to differential N-linked glycosylation of the transporter protein. These findings support other studies using site-directed mutagenesis of Asn^{45} to induce defective GLUT1 glycosylation, which causes entrapment of GLUT1 within the intracellular compartment [2]. In addition to GLUT1 carbohydrate, GLUT1 sequences within the polypeptide chain may also participate in subcellular localization of the transporter protein [22]. Such sequences may interact with membrane protein recognition sites that are selectively localized to the apical or basolateral membrane within a barrier cell. The hypothesis that the subcellular localization of GLUT1 at the BBB is controlled in part through differential glycosylation may be tested in future studies that examine the mobility on SDS-PAGE of GLUT1 selectively isolated from lumenal versus ablumenal brain capillary endothelial membranes [31].

Acknowledgments

This work was supported by National Institutes of Health Grant P01-NS-25554, by National Institutes of Health Training grant T32-NS-07356 (K.J.D.), and by a Juvenile Diabetes Foundation Fellowship (A.K.K.). The authors thank Dr. Ruben J. Boado for numerous invaluable discussions and Ms. Jing Yang for expert technical assistance.

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