GLUT1 Glucose Transporter Expression in the Diabetic and Nondiabetic Human Eye

Arno K. Kumagai,* Ben J. Glasgow,†‡ and William M. Pardridge*§

Purpose. The GLUT1 glucose transporter is expressed in endothelial and epithelial barriers, including the retinal capillary endothelium and the retinal pigment epithelium (RPE) of the eye. The present studies were undertaken to determine whether GLUT1 is expressed in additional cell types within the human eye and whether retinal endothelial GLUT1 is aberrantly expressed in diabetic proliferative retinopathy in humans.

Methods. Immunohistochemical staining of sections of human eyes obtained at surgery or autopsy from patients with and without diabetes was performed with polyclonal antisera directed against the human GLUT1 glucose transporter.

Results. In the course of this study, an unexpected multicellular localization of GLUT1 in different cellular barriers of the human eye was observed. In the nondiabetic eye, specific staining for GLUT1 was seen in the nerve fiber layer, the ganglion and photoreceptor cell bodies, the capillaries and the RPE of the retina, the basal infoldings of the pigmented and nonpigmented layers of the ciliary body, the capillary endothelium and posterior epithelium of the iris, the corneal epithelium and endothelium, and the endothelium lining of the canal of Schlemm. Müller cells, a type of retinal glial cell identified by morphology and by parallel staining for glial fibrillary acidic protein, also stained intensely positive for GLUT1. The pattern of GLUT1 immunoreactivity in the diabetic eyes was virtually identical to that in the nondiabetic specimens, with the notable exception that the neovascular endothelium of proliferative diabetic retinopathy did not stain for GLUT1.

Conclusions. These studies describe the heretofore unrecognized expression of immunoreactive GLUT1 in the ganglion cell layer of the retina, the endothelium lining the canal of Schlemm, the corneal endothelium, and the basal cells of the corneal epithelium of the human eye. The present study also provides evidence for immunoreactive GLUT1 in glial cells of the central nervous system. Because the expression of GLUT1 is characteristic of tissues that possess a barrier function, the absence of GLUT1 immunoreactivity in the neovascular tissue of proliferative diabetic retinopathy suggests that the loss of selective permeability is associated with an absence of facilitated glucose transport in this disorder. Invest Ophthalmol Vis Sci. 1994;35:2887–2894.

States of acute and chronic hyperglycemia give rise to pathophysiologic processes in various structures of the eye. Within these structures, glucose gradients are thought to be maintained by isoforms of the sodium-independent glucose transporter gene family.1 GLUT1, the first such isoform to be cloned,2 is expressed in high density on the membranes of human erythrocytes and on the brain capillaries that comprise the blood–brain barrier.3 In addition to the blood–brain barrier, GLUT1 is expressed in other tissues possessing barrier functions, including the endothelia of the retinal capillaries of the eye.4,5 Because the neovascularization of the retina found in proliferative diabetic retinopathy is characterized by “leaky” retinal vessels (that is, by a loss of barrier function),6 we investigated whether GLUT1 expression is preserved in the neovascularized diabetic retina. A second goal of the present study was to examine the expression of the

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GLUT1 glucose transporter in different cellular barriers of the nondiabetic human eye. To date, virtually all immunohistochemical investigations demonstrating GLUT1 in the eye have shown findings made in animals. The present studies demonstrate that the distribution of the GLUT1 glucose transporter in the human eye is more extensive than that previously observed in other mammals.

RESEARCH DESIGN AND METHODS

Primary Antisera
Polyclonal antisera against either purified human erythrocyte glucose transporter or against a synthetic peptide corresponding to the last 13 amino acids of the carboxyl terminus of the human brain–erythrocyte glucose transporter were raised by serial inoculations in New Zealand rabbits, as described previously, and were designated anti-hGT and anti-CT, respectively. Polyvalent rabbit antiserum against bovine glial fibrillary acidic protein (GFAP) was purchased from Dako (Carpenteria, CA).

Preparation of Eye Sections
Formalin-fixed, paraffin-embedded specimens of human eyes that had been recovered at autopsy from patients with and without diabetes were obtained from the archives of the Jules Stein Eye Institute at UCLA. Additional specimens obtained at autopsy or surgery were generously provided by Dr. Morton E. Smith, (Washington University, St. Louis, MO). Five nondiabetic and 13 diabetic eyes, one eye per patient, were studied. Five micron sections were cut from each eye block and transferred to coated microscope slides (Fisher Scientific Products, Tustin, CA). The sections were incubated overnight at 37°C, heat-fixed at 52°C for 15 minutes, deparaffinned by treatment with acetone, and rehydrated with serial dilutions of ethanol. Endogenous peroxidases were removed by treatment with 0.3% H2O2, and nonspecific binding was decreased by incubation with 3% goat serum at room temperature for 30 minutes.

Immunohistochemical Staining
Eye sections were incubated 90 minutes at room temperature with either anti-hGT, anti-CT, or anti-GFAP
GLUT1 Expression in the Human Eye

antiserum were stained with identical dilutions of preimmune serum (for anti-hGT, anti-GFAP) or with anti-CT antiserum that had been preabsorbed with synthetic peptide. After incubation with the primary antiserum, three 5-minute washes in TBS were performed, followed by incubation at room temperature for 30 minutes with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a concentration of 50 μg/ml in TBS. Three 5-minute washes in TBS were repeated, followed by treatment with an avidin-biotin-peroxidase complex (ABC Elite, Vector) for 30 minutes. After a washing procedure identical to those described above, the eye sections were developed for 15 to 20 minutes at 37°C in peroxidase substrate, which consisted of 0.02 M sodium acetate (pH 5.1), 0.17 mg/ml 3-amino-9-ethylcarbazole (AEC, Sigma, St. Louis, MO), 10% dimethyl sulfoxide, and 0.20% H2O2. The slides were briefly counterstained with Mayer’s hematoxylin (Sigma), mounted with glycerol, and viewed under light microscopy.

Semi-quantitative analysis of the intensity of the reaction product seen in the various eye structures studied was performed. The intensity of GLUT1 immunostaining of the various structures was compared to the staining intensity of structures known to express GLUT1. The reference structures employed in this scoring system were: 1+ = the human erythrocyte, 2+ = the cytoplasm of the nonpigmented layer of the ciliary body, and 3+ = the basolateral surface of the nonpigmented layer of the ciliary body. The grading system allowed for a convenient internal reference for each slide in which the reference structures were present. An individual score was assigned to each eye structure studied, which ranged from 0 (total absence of staining) to 3+ (most intensely stained). The accuracy of this scoring system was tested by an independent review of the slides by an observer who was masked to the previous staining intensity scores, the use of anti-GLUT1 or preimmune antiserum, and the presence or absence of diabetes in the patients from whom the specimens were obtained. In addition, a masked comparison was made between the staining patterns of adjacent sections of eye specimens stained with either anti-hGT or anti-CT antiserum or with the respective control antisera (preimmune antiserum or anti-CT antiserum preabsorbed with CT peptide, respectively). Between 8 and 12 slides per specimen were studied.

RESULTS

The clinical data for all patients without diabetes and for all patients with diabetes for whom data were available (6 of 13) are given in Tables 1 and 2. A list of the eye structures studied in both diabetic and nondiabetic specimens, with the respective relative intensity staining scores, is presented in Table 3. The relative scores made by the investigators and a masked observer were virtually identical for all structures listed. The staining patterns were consistent among all specimens examined, and slight variations in staining could be attributed to pre-embedding necrosis or artifact. With the notable exception of the staining pattern of the preretinal vessels of the diabetic eyes with proliferative diabetic retinopathy (PDR, discussed below), the

<table>
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<th>Structure</th>
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<tr>
<td>Retina</td>
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<td>Inner limiting membrane*</td>
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<td>Ganglion cell bodies*</td>
<td>1-2+</td>
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<td>Cell bodies of rods and cones*</td>
<td>2+</td>
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<tr>
<td>Outer limiting membrane*</td>
<td>2-3+</td>
</tr>
<tr>
<td>Outer segments, rods and cones*</td>
<td>0</td>
</tr>
<tr>
<td>Retinal pigmented epithelium (a, b)</td>
<td>3+</td>
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<tr>
<td>Molar body</td>
<td></td>
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<tr>
<td>Pigmented epithelium</td>
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<tr>
<td>Nonpigmented epithelium</td>
<td>3+ (R)</td>
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<td>Endothelium*</td>
<td>3+</td>
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<tr>
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<td>0</td>
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<tr>
<td>Cornea</td>
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<tr>
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<td>1-3+ (var.)</td>
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<tr>
<td>Endothelium (a, b)*</td>
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<td>Lens</td>
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<td>Epithelium*</td>
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<td>Cortex and nucleus*</td>
<td>0</td>
</tr>
<tr>
<td>Human erythrocyte</td>
<td>1+ (R)</td>
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* New findings of this study.

Semi-quantitative scoring of staining intensity of immunoperoxidase reaction product from immunocytochemical staining of 7-μm sections of human diabetic and nondiabetic eyes with polyclonal antiserum directed against purified human erythrocyte glucose transporter. The scoring system was based on the relative intensity of staining of eye structures in comparison to reference structures (R) in which GLUT1 expression is well documented.5,6 The grading scale reflects total absence of staining (0) to most intense staining (3+). The reproducibility of the scale was verified by masked comparison. a, apical membrane; b, basolateral membrane.
distribution and intensity of GLUT1 immunoreactivity in various eye structures were virtually identical between the nondiabetic and diabetic specimens. This finding was confirmed by masked observation. Furthermore, no significant difference could be detected on masked comparison between specimens stained with either anti-hGT or anti-CT GLUT1 antisera.

In the retina, intense specific immunostaining for GLUT1 (2 to 3+) was seen in the nerve fiber layer, the Müller cells, and the outer limiting membrane, which separates the photoreceptor cell bodies from the more distal inner and outer segments (Fig. 1A; individual retinal layers are identified in Fig. 1B, right panel). Less intense (2+) but specific staining was also observed in the ganglion cell layer and in the cell bodies of the rods and cones (Fig. 1A). In confirmation of previous findings, the endothelia of retinal capillaries and the apical and basolateral surfaces of the retinal pigment epithelium (RPE) stained intensely (2 to 3+) for GLUT1 (Fig. 1A); in contrast, the endothelia of the choriocapillaris did not stain (Fig. 1A, large arrowhead). The inner and outer segments of the photoreceptor cells (Fig. 1A, small arrow), as well as the inner limiting membrane (Fig. 1A, large arrow) did not show GLUT1 immunoreactivity. Sections treated with either preimmune serum or anti-CT antiserum preabsorbed with CT synthetic peptide showed an absence of staining in all structures of the eye (Fig. 1B, right panel).

Müller cells, a type of retinal glia, are oriented radially and possess cytoplasmic processes that span the retina between the inner and outer limiting membranes. On the basis of their unique morphology and orientation, the Müller cells could be distinguished within the immunopositive retina. These cells stained intensely for GLUT1 in both diabetic and nondiabetic eyes (Fig. 1D).

Müller cells have numerous dendritic processes that contact and encircle various structures within the retina, including the neuronal axons of the nerve fiber layer, retinal vessels, and the cell bodies of the ganglion and photoreceptor cells. Because of these characteristics, it was difficult to determine whether the axons of the nerve fiber layer and the ganglion and photoreceptor cell bodies themselves stained for GLUT1, or whether the immunoreactivity in these structures was due to overlying or surrounding retinal glial cell dendritic processes. To clarify this issue, adjacent sections of nondiabetic retina were stained with anti-GLUT1 (Fig. 1A, right panel) and anti-GFAP antisera (Fig. 1B, left panel). As can be seen in Figures 1A and 1B, although Müller cells and ganglion and photoreceptor cell bodies stained for GLUT1, immunoreactivity for GFAP could be clearly detected only in the radial extensions of the Müller cells and in the nerve fiber layer. This differential staining pattern supported initial impressions that the cell bodies of the ganglion cells and photoreceptor elements expressed GLUT1. It could not be determined, however, whether or not the staining of the axons in the nerve fiber layer was due to staining of glial elements.

In the cornea, specific staining for GLUT1 was also observed in the apical and basolateral surfaces of the suprabasal epithelial layer and of the corneal endo-

FIGURE 1. Immunocytochemical staining of human eyes with anti-GLUT1 or anti-GFAP antisera. (A) GLUT1 immunoreactivity in cross-section of nondiabetic whole retina (left panel) or inner layers of retina (right panel). Large arrow, inner limiting membrane; small arrow, distal segments of photoreceptor cells; hollow arrow, retinal capillary endothelium; large arrowhead, choriocapillaris with GLUT1 positive erythrocytes. Specific retinal layers are identified in (B), right panel. Bar in left panel, 30 μm; bar in right panel, 20 μm. (B), left panel. GFAP immunostaining of eye section adjacent to that shown in (A), right panel. Bar, 20 μm. Right panel, control slide of nondiabetic retina stained with preimmune serum; (1) inner limiting membrane; (2) nerve fiber layer; (3) ganglion cell layer; (4) inner plexiform layer; (5) bipolar cell layer; (6) outer plexiform layer; (7) layer of the photoreceptor cell bodies; (8) external limiting membrane; (9) retinal pigment epithelium. v, vitreous; c, choroid. Bar, 30 μm. (C) Left panel, GLUT1 immunostaining of diabetic retina with proliferative retinopathy containing extraretinal vessels. Erythrocytes within the lumen of the extraretinal vessels stain positively for GLUT1. Right panel, control slide from same eye stained with preimmune serum. Bar, 30 μm. (D) GLUT1 immunostaining of slightly disrupted nondiabetic retina demonstrating positive staining of Müller cells. v, vitreous. Bar, 20 μm. (E) GLUT1 immunostaining of nondiabetic eye showing canal of Schlemm (hollow arrow); ciliary body (Cb) with staining of basilar infoldings of nonpigmented cell layer (small arrows); and iris (I). AC, anterior chamber. Bar, 125 μm. Inset, high-power magnification of endothelial lining of canal of Schlemm. Bar, 20 μm. F. GLUT1 immunostaining of nondiabetic cornea. Right panel, low-power magnification. Large arrow, corneal epithelium; small arrow, corneal endothelium; S, stroma; AC, anterior chamber. Bar, 125 μm. Insets, high-power magnification of corneal epithelium (upper) and endothelium (lower). Bar, 10 μm.
The findings of the present study are consistent with studies of GLUT1 localization to both endothelial (retinal and iridal) and epithelial (ciliary body, RPE, iris) barriers of the eye (Table 3). Second, we report new findings on GLUT1 distribution in additional endothelial and epithelial barriers of the human eye, including the endothelium of the canal of Schlemm, the corneal endothelium, and the basal layer of the corneal epithelium (Table 3). Third, the presence of abundant immunoreactive GLUT1 protein is demonstrated in a type of retinal glia, the Müller cell (Fig. 1D). Fourth, GLUT1 immunoreactivity is shown to be absent in the extraretinal vessels of proliferative diabetic retinopathy (Fig. 1C).

In the retina, GLUT1 immunoreactivity is seen in the nerve fiber layer, the ganglion cell layer, the outer nuclear layer containing the photoreceptor cell bodies, the outer limiting membrane, and the RPE (Fig. 1A). The cell bodies of both ganglion cells and photoreceptor elements appeared to express immunoreactivity for GLUT1, as was shown in the colocalization experiment with Müller cells employing anti-GFAP and anti-GLUT1 antisera (Fig. 1A and 1B).

An unexpected finding in the present study is the demonstration of GLUT1 immunoreactivity in the retinal Müller cells (Fig. 1D). Although GLUT1 protein has been demonstrated in primary cultures of rat brain glial cells and GLUT1 mRNA is expressed in human high-grade gliomas, no immunohistologic studies to date have shown the presence of GLUT1 in normal, mature central nervous system glial cells. Because the retina is a direct extension of the central nervous system, the present study documents GLUT1 expression in a type of central nervous system glia. Because of their unique radial orientation and extensive dendritic processes, Müller cells are thought to serve as the principal nutritional supporting cells of the mammalian retina. In situ staining for immunoreactive GLUT1 protein in retinal Müller cells lends support to this hypothesis because glucose represents the chief metabolic substrate of the retina.

The staining of the corneal epithelium demonstrates immunoreactivity of the basolateral and apical surfaces of the suprabasal cell layer, with attenuation in the mature epithelial cells at the corneal surface (Fig. 1F, upper inset). This pattern of GLUT1 expression in corneal epithelium coincides with the localization of intercellular tight junctions at the apices of the suprabasal cell layer. An identical staining pattern for GLUT1 has been noted in human epidermis. Expression of GLUT1 protein in the corneal endothelium (Fig. 1F, lower inset), as with its expression in corneal epithelium, allows for regulation of glucose transport across these cells layers and into the avascular stroma. With regard to the endothelium lining Schlemm’s canal (Fig. 1E), a possible role for GLUT1 in the osmotic regulation of the aqueous humor as it enters the venous system is suggested by the differences in osmolality between aqueous humor and plasma and the observation that GLUT1 may act as a water channel in tissues in which it is expressed.

The lack of immunoreactivity for GLUT1 in the lens supports binding studies employing cytochalasin B, a specific ligand for glucose transport systems. These studies demonstrate low specific binding of cytochalasin B in the human lens. Although hyperglycemia initiates both acute swelling and chronic cataract formation, it is unclear at present how glucose is transported into the interior of the lens via mechanisms other than free diffusion.

One of the earliest manifestations of the neovascularization characteristic of proliferative diabetic retinopathy (PDR) is the breakdown of the blood–retinal...
GLUT1 Expression in the Human Eye

2893

barrier (BRB), which is evidenced clinically by the leakage of fluorescein into the vitreous during fluorescein angiography.22 The BRB consists of two major components: the endothelial cells of the retinal capillaries (the inner BRB) and the cells of the retinal pigment epithelium (the outer BRB), the barrier functions of which are conferred by tight junctions (zonulae occludens) between adjacent endothelial or epithelial cells.22 Although not settled conclusively, it appears that the compromise of BRB integrity associated with PDR occurs principally at the level of the retinal capillary endothelial cells.24

In the present study, we demonstrate the absence of immunoreactive GLUT1 protein in the neovascular tissue characterizing PDR (Fig. 1C). Previous investigators have suggested that GLUT1 glucose transporter expression is a marker of tissues that serve a barrier function.4,5 The results of the present study suggest a corollary to this hypothesis: In tissues in which there is a pathologic loss of barrier function, such as the endothelia of the neovascular tissue of PDR, there is an associated absence of immunoreactive GLUT1 transporter expression. The observation made in these studies that immunoreactive GLUT1 transporter expression is absent in both preretal neovascular fronds and in more mature preretal fibrovascular membranes suggests that absence of GLUT1 expression is not characteristic of neovascular tissue per se but is associated with the permanent loss of the barrier properties seen in the pathologic vessels of PDR. At present, it is unclear whether the absence of GLUT1 expression is restricted to the proliferative vessels themselves or is characteristic of the retinal vascular changes of nonproliferative diabetic retinopathy as well. Further investigations of GLUT1 transporter expression in early diabetic retinopathy may clarify this issue.

Key Words

GLUT1, glucose transporter, retinopathy, blood-retinal barrier, Müller cells, glia, neovascularization

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