

# Diabetes Downregulates GLUT1 Expression in the Retina and Its Microvessels but Not in the Cerebral Cortex or Its Microvessels

Gamal A. Badr, Jie Tang, Faramarz Ismail-Beigi, and Timothy S. Kern

Capillaries in the retina are more susceptible to develop microvascular lesions in diabetes than capillaries in the embryologically similar cerebral cortex. Because available evidence implicates hyperglycemia in the pathogenesis of diabetic retinopathy, differences in glucose transport into the retina and brain might contribute to this observed tissue difference in susceptibility to diabetes-induced microvascular disease. Thus, we compared levels of GLUT1 and GLUT3 expression in the retina, cerebrum, and their respective microvessels by Western blot analysis. In nondiabetic animals, the content of GLUT1 protein in retina and its microvessels was multifold greater than that of cerebral cortex gray matter and its microvessels. Streptozotocin-induced diabetes of a 2-week or 2-month duration reduced GLUT1 expression in the retina and its microvasculature by ~50%, but it resulted in no reduction in GLUT1 expression in cerebrum or its microvessels. The density of capillaries in retinas of diabetic animals did not change from normal, and so the observed decrease in GLUT1 expression in the retina and retinal capillaries of diabetic animals cannot be attributed to fewer vessels. Despite the diabetes-induced reduction of GLUT1 expression in retina, neural retina of diabetic rats still possessed more GLUT1 than the cerebrum. Retinal pigment epithelium (RPE) possessed more GLUT1 than neural retina or its microvessels, and expression of the transporter in the RPE was not affected by diabetes. GLUT3 levels were greater in cerebral gray matter than in retina, and they were unaffected by diabetes in either tissue. The effect of diabetes on GLUT1 expression differs between retina and cerebral cortex, suggesting that glucose transport is regulated differently in these embryologically similar tissues. Because diabetes results in downregulation of GLUT1 expression in retinal microvessels, but not in RPE, the fraction of the glucose entering the retina in diabetes is likely to be greater across the RPE than across the retinal vasculature. *Diabetes* 49:1016–1021, 2000

**H**yperglycemia is sufficient to initiate the development of diabetic retinopathy. This fact has been made especially clear by the development of diabetic-like retinopathy in normal nondiabetic dogs and rodents that were experimentally made hyperglycemic by feeding them a diet enriched with galactose (1–5). Studies demonstrating that intensive therapy sufficient to minimize hyperglycemia inhibits the development of retinopathy (6–8) are consistent with the evidence that hyperglycemia plays a critical role in the pathogenesis of retinal disease. However, the mechanism by which hyperglycemia causes diabetic retinopathy remains unclear. Excessive transport or concentration of glucose within cells of the retina is a common thread underlying most of the biochemical mechanisms that have been postulated to play a role in the pathogenesis of diabetic retinopathy.

Retina and cerebral cortex are embryologically similar, yet, we and others (9–11) have found them to differ in their susceptibility to develop microvascular lesions of diabetes. Unlike the extensive microvascular pathology that develops in retinal capillaries in diabetes, cerebral capillaries show no increase in the number of acellular capillaries, microaneurysms, or pericyte ghosts (11). Because both vascular beds are exposed to similar concentrations of blood hexose, these observations suggest that factors in addition to systemic blood glucose concentration influence the development of retinal vascular disease in diabetes.

Transport of glucose into cells can vary among cell types, and such differences might be important in determining which cells are adversely affected by hyperglycemia. Transport of glucose into retina and brain occurs across the blood-retinal and blood-brain barriers via the GLUT1 transporter. Effects of diabetes on GLUT1 expression in cerebral cortex and its microvessels have been examined by several investigators, but the results have been controversial (12–16). In contrast, effects of diabetes on GLUT1 expression in retinal microvessels have not been studied as thoroughly (17). In the present study, retina and cerebral cortex, their respective microvessels, and retinal pigment epithelium (RPE) have been directly compared with respect to the effects of diabetes on GLUT1 expression.

## RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats weighing 225–250 g were used in this study. Rats were randomly assigned to become diabetic or to remain nondiabetic controls. Diabetes was induced by the injection of a freshly prepared solution of streptozotocin in citrate buffer (pH 4.5) at a dose of 60 mg/kg body wt in the tail vein.

From the Departments of Medicine, Ophthalmology, and Physiology and Biophysics, and the Center for Diabetes Research, Case Western Reserve University, Cleveland, Ohio.

Address correspondence and reprint requests to Timothy S. Kern, PhD, Clinical and Molecular Endocrinology, Case Western Reserve University, Cleveland, OH 44106-4951. E-mail: tsk@po.cwru.edu.

Received for publication 21 December 1998 and accepted in revised form 17 February 2000.

G.A.B. and J.T. contributed equally to this work.

RPE, retinal pigment epithelium; TBS, Tris-buffered saline; TBST, Tris-buffered saline containing Tween.

After 1 week, a sample of blood was obtained from the tail vein for measurement of the serum glucose concentration to verify the presence of hyperglycemia. Glycated hemoglobin levels were measured at 2 months by affinity chromatography (Glyc-Affin; Pierce, Rockford, IL) after an overnight fast. Insulin levels were measured in representative animals by a double-antibody radioimmunoassay (18). Our treatment of the animals conformed to the resolution of the Association for Research in Vision and Ophthalmology on the treatment of animals in research.

Rats were killed at the indicated times by CO<sub>2</sub> inhalation anesthesia followed by decapitation. For the 2-week study, cerebral cortex and retinas were isolated, frozen in dry ice, and stored at -80°C until used. For the 8-week study, control and diabetic rats were divided into replicate groups (2–3 rats per replicate). Retinal and cerebral microvessels were isolated as later described for Western blot analysis of GLUT1. After removal of pia, a slice of gray matter (based on gross appearance of thin slices of cerebrum that did not encompass more than the outer 5 mm of cerebrum from the frontal lobe) and whole retina were used for quantitation of whole-tissue GLUT1 by Western blot. Segments of frontal lobe, hippocampus, and retina were saved from each replicate group for quantitation of vascular density. After removal of neural retina, RPE was isolated by the method described by Cingle et al. (19), in which 1 mmol/l EDTA was added to the eyecup for 20 min, followed by gentle brushing to dislodge the RPE cells.

Samples of brain, retina, and RPE were disrupted using a Potter-Elvehjem homogenizer (1:15 dilution) in a solution containing 0.25 mol/l sucrose, 10 mmol/l Tris, and 0.5 mmol/l EGTA, pH 7.4. Homogenates were centrifuged at 2,000g for 5 min, and the resulting postnuclear supernatants were used in Western blot analysis. It is possible that some intact vessel fragments were pelleted by this procedure, but in other samples, lower speed (500g) or sonication to totally disrupt all cells did not alter results or conclusions. For the cerebrum, up to 50 and 20 µg protein were loaded per lane in blots to be reacted with anti-GLUT1 and anti-GLUT3 antibodies, respectively. For the retina, up to 30 and 75 µg protein were loaded per lane in blots for analyses of GLUT1 and GLUT3, respectively. RPE was loaded at a concentration of 2 µg for analysis of GLUT1. Samples were solubilized in SDS and subjected to SDS-PAGE (12% acrylamide gels). After SDS-PAGE and the transfer to a nitrocellulose membrane (BAS-85; Schleicher and Schuell, Keene, NH), blots were incubated in Tris-buffered saline (TBS) (20 mmol/l Tris and 137 mmol/l NaCl, pH 7.6) containing 0.1% Tween 20 (TBST) and 5% nonfat milk. After 3 washes of each for 10 min in TBST, the blots were incubated with 1:300 dilution of affinity-purified rabbit anti-GLUT1 or 1:10,000 dilution of anti-GLUT3 IgG in TBST for 1 h. After 3 washes with TBST, blots were reacted for 1 h with 1:3,000 dilution of peroxidase-labeled goat anti-rabbit IgG (Gibco BRL, Gaithersburg, MD) in TBST containing 0.15% nonfat milk and were then washed 3 times with TBS devoid of the detergent and were developed using an enhanced chemiluminescence kit (Amersham Life Science, Buckinghamshire, U.K.). Films were digitized, and the intensity of the bands was quantitated using OS-Scan Image Analysis software (USB State Biochemical, Cleveland, OH). Tests that used either purified erythrocyte GLUT1 or erythrocyte cell membrane indicated that the methods used were linear in the range of interest. Antibodies against the glucose transporters were obtained from Charles River Pharmaservices (Southbridge, MA).

Several steps were taken to ensure that comparable amounts of protein had been loaded onto each lane of the gels. After staining with anti-GLUT1, membranes were destained and then restained with Ponceau S staining solution (Sigma, St. Louis, MO). The intensities of the representative bands were visually similar across the lanes. Results were not regarded as reliable unless the intensities of multiple bands were judged to be comparable among the lanes. To further assess the validity of the conclusions derived from this method, other membranes were stripped after staining with anti-GLUT1 antibody and were then restained with antibody against Na,K-ATPase ( $\alpha$ 1 subunit) (obtained from the University of Iowa Hybridoma facility), GAPDH (Chemicon, Temecula, CA), or actin (Sigma). Bands were quantitated by scanning densitometry. Calculating GLUT1 expression relative to intrinsic proteins, such as Na,K-ATPase, GAPDH, and actin, was found to be useful in assessing the effect of diabetes on GLUT1 within a tissue; however, it was not as useful for making comparisons among tissues, because the different tissues were found to have different amounts of each of the intrinsic proteins. Of the intrinsic proteins examined, GAPDH expression was found to differ least between retina and cerebral cortex.

**Isolation of microvessels.** Microvessels from cerebral cortex gray matter were isolated using previously published methods (20,21) with some modifications. Briefly, brains from 3 rats were rapidly removed and immersed in ice-cold Earl's-HEPES buffer containing Earl's salts, 20 mmol/l HEPES (pH 7.4), and 1% bovine serum albumin. The brain stem and cerebellum were dissected away and discarded, and the pia and other large superficial blood vessels were removed using fine forceps. The gray matter was isolated using a sharp razor blade and minced with fine scissors. The small pieces of gray matter were homogenized by hand (3–4 strokes) in 30 ml of the previously described

buffer by using a Dounce homogenizer (Fisher, Pittsburgh, PA) with a loosely fitting pestle. The homogenate was sieved through 210 µm nylon mesh, and the material collected on the mesh was homogenized again in 20 ml of the buffer using 2–3 strokes. The homogenate was sieved through 105 µm nylon mesh, and microvessels collected on the mesh were washed gently with 10 ml of the buffer and were then collected. The material was layered over 5 ml of 1.3 mol/l sucrose containing 10 mmol/l Tris (pH 7.4) and was centrifuged at 10,000g for 10 min at 4°C. The pellet containing purified microvessels was washed twice with ice-cold phosphate-buffered saline. The purity of each microvessel preparation was monitored by light microscopy and was similar in control and diabetic groups. We estimated that contaminants (mostly naked nuclei) did not exceed 5% of the nuclei in the preparation and that the yield of microvessels from both experimental groups was equal (~60 µg protein).

The recovery of microvessels from pooled retinas from 3 rats by using the sieving method previously described was poor. Thus, retinal vasculature was isolated from whole retina by an osmotic method that we have used previously (22). Briefly, freshly isolated retina were incubated in distilled water for 1 h, followed by a 5-min incubation with DNase I (2 mg/ml). The retinal microvasculature was isolated under microscopy by repetitive inspiration and ejection through Pasteur pipettes with sequentially narrower tips. Retinal microvessels isolated by this method showed a normal complement of nuclei and were devoid of all nonvascular materials.

The microvessels were prepared for Western blots, as previously described, except they were sonicated briefly in SDS sample buffer to assist in solubilization. Protein was measured by Micro BCA reagent (Pierce), and Western blots were prepared using 5 µg protein/sample as previously described.

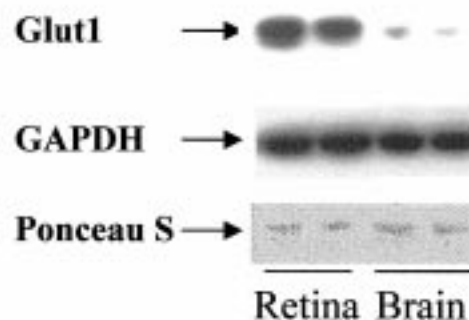
The density of microvessels in the retina and the gray matter of the frontal lobe was quantitated in paraffin sections using point-counting methods (23). Hematoxylin and eosin-stained sections were examined at  $\times 250$  magnification, and the fraction of grid points (10  $\times$  10 grid) intersecting with capillary cross-sections was counted. For frontal lobes, the density of capillary profiles was calculated by averaging 4 different regions per tissue section (total area of 0.67 mm<sup>2</sup>). The density of capillaries in retinal cross-sections was estimated as the number of capillary profiles observed per 40 mm linear length of retina. Because the number of microvessels counted in retinal cross-sections was modest, the density of retinal microvessels was also assessed in Periodic acid-Schiff-stained flat preparations of isolated retinal vasculature. The 2 methods used for measurement of retinal vascular density yielded similar conclusions.

Results are expressed as means  $\pm$  SE. Student's unpaired 2-tailed *t* test was used, and a *P* < 0.05 was considered significant.

## RESULTS

Diabetic rats were hyperglycemic and insulinopenic, and they failed to gain weight at a normal rate. Body weight at 8 weeks averaged 387 and 256 g for normal and diabetic rats, respectively. Serum glucose levels ( $4.4 \pm 0.7$  and  $21.7 \pm 4.3$  mmol/l for normal and diabetic groups, respectively) and glycosylated hemoglobin concentrations ( $4.7 \pm 0.2$  and  $12.2 \pm 0.6\%$ ) were greater than normal in diabetic rats. Serum insulin levels in diabetic rats were low ( $0.4 \pm 0.1$  ng/ml) compared with those in controls ( $2.5 \pm 0.2$  ng/ml).

The content of GLUT1 protein in retina of normal rats was greater than that in cerebral cortex gray matter; retina pos-



**FIG. 1.** GLUT1 expression in retina of nondiabetic rats is greater than that in cerebral gray matter. GAPDH expression and Ponceau S staining of protein on the same gel are included to demonstrate comparable loading of lanes.

essed 6 times more of the transporter than cortex (Fig. 1). In homogenates of retina and cerebrum, the transporter was about 45 kDa, and retina showed a wider band of staining than cerebrum. Diabetes of a 2-week (data not shown) or 8-week (Fig. 2) duration significantly reduced GLUT1 content of retina by ~33 and 50%, respectively. In contrast, GLUT1 in cerebral cortex gray matter was not reduced by diabetes at either time point (Fig. 2).

Microvessels from both retina and cerebrum had the 55-kDa isoform of GLUT1 (Fig. 3). Consistent with findings in whole tissues, the content of GLUT1 in microvessels isolated from retinas of nondiabetic rats was multifold greater than that in cerebral microvessels. Diabetes reduced GLUT1 levels in retinal microvessels by >60% compared with nondiabetic rats, whereas expression in cerebral microvessels did not decrease.

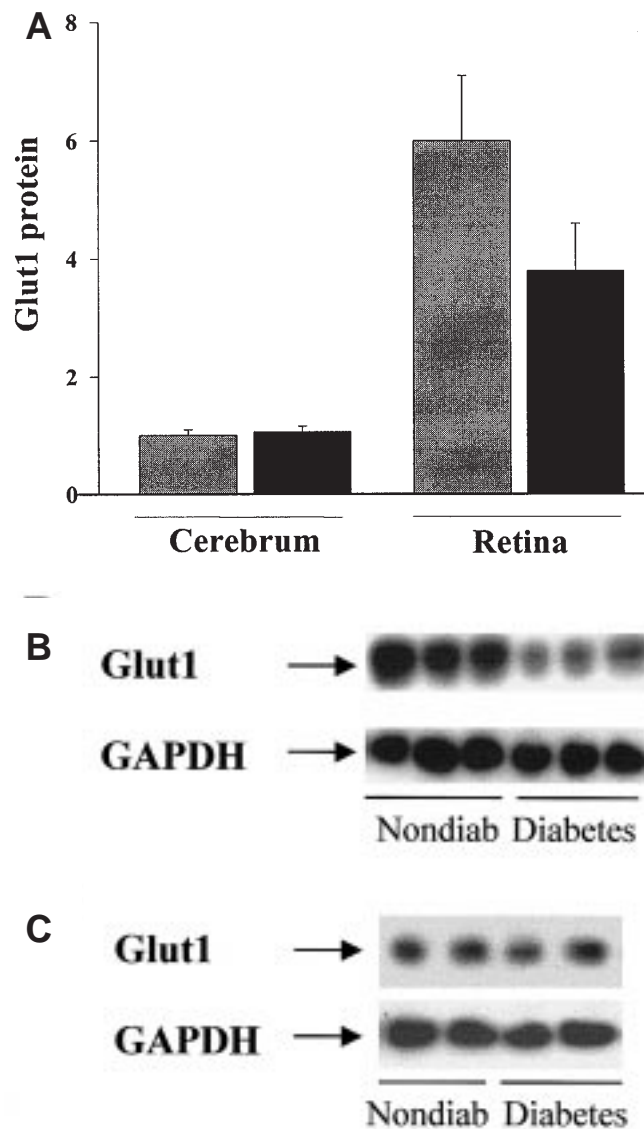


FIG. 2. Diabetes of an 8-week duration results in subnormal expression of the glucose transporter in retina, whether expressed as relative to the protein loaded (A) or the GAPDH expression (B). Diabetes did not decrease GLUT1 in cerebral gray matter (A and C). The y-axis in A is *n*-fold the value of nondiabetic cerebral gray matter. Data are means  $\pm$  SE (*n* > 8 per group). □, Nondiabetic rats; ■, diabetic rats. Nondiab, nondiabetic.

Diabetes of a 2-month duration did not significantly alter the microvascular density in either retina (normal  $7.9 \pm 0.5$  capillaries/40 mm retina, diabetes  $7.9 \pm 0.7$  capillaries/40 mm retina) or the brain (normal  $5.0 \pm 0.5$  capillaries/mm<sup>2</sup>, diabetes  $5.9 \pm 0.4$  capillaries/mm<sup>2</sup>), indicating that the observed loss of retinal GLUT1 in diabetes was not due to a change in vascular density. RPE had a multifold greater expression of GLUT1 than neural retina and cerebrum, and expression of the transporter in RPE was not affected by diabetes (Fig. 4).

The content of GLUT3 was ~12 times greater in cerebral cortex gray matter than in retina (Fig. 5). In contrast to GLUT1, the content of GLUT3 was unaffected by diabetes in either tissue.

**DISCUSSION**

Elevation of systemic levels of glucose or galactose in diabetes or experimental galactosemia can induce a variety of abnormalities of metabolism of the retina and its microvasculature, some of which, presumably, contribute to the development of retinopathy. To date, however, the mechanism by which hyperglycemia causes retinopathy remains unclear. Abnormalities that have attracted interest as potential causes of retinopathy include excessive polyol pathway activity, non-enzymatic glycation, oxidative stress, and stimulation of reti-

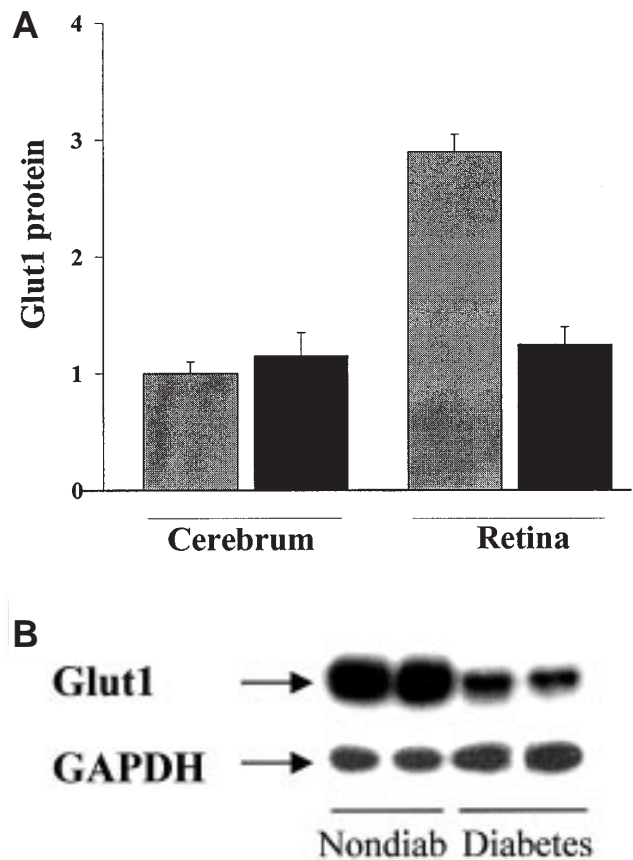


FIG. 3. GLUT1 expression in microvessels isolated from retina, but not in microvessels from cerebral tissue of the same animals, is decreased by diabetes of an 8-week duration. Graphical summary (A) and raw Western blots (B) are provided. The y-axis in A is *n*-fold the value of nondiabetic cerebral microvessels. Data are means  $\pm$  SE (*n* = 6 per group). □, Nondiabetic rats; ■, diabetic rats. Nondiab, nondiabetic.



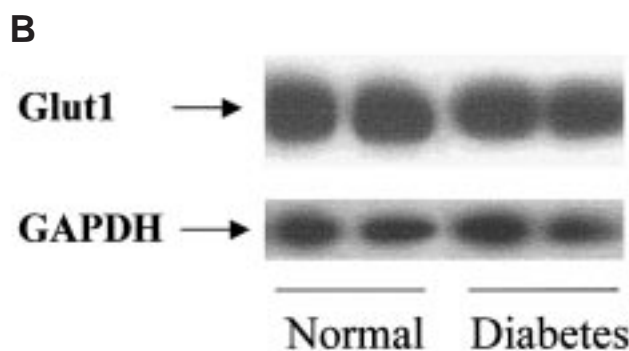
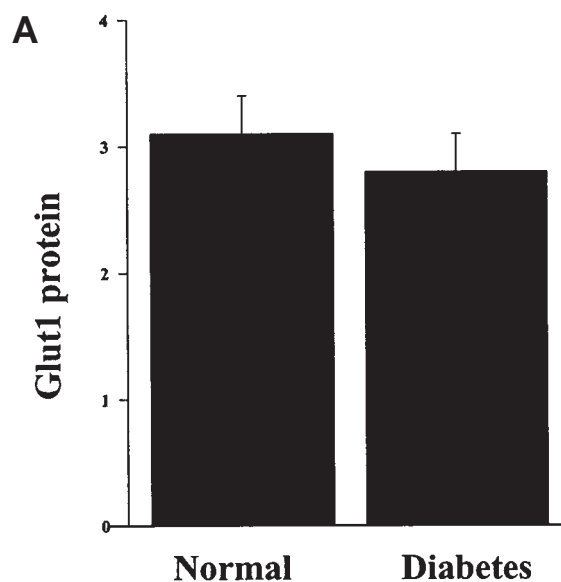


FIG. 4. Diabetes of an 8-week duration does not alter GLUT1 expression in RPE, whether expressed as relative to the microgram of protein loaded (A) or relative to GAPDH expression (B).

nal protein kinase C activity (22,24–32). Most of these metabolic abnormalities are dependent on excessive transport of hexose into retinal cells or increased concentration of hexose within those cells.

Diabetic retinopathy is generally regarded to primarily affect the microvasculature; therefore, metabolic abnormalities of microvascular cells have been of special interest. Endothelial cells apparently differ from many other cell types in that transport of glucose into the cells is more rapid than metabolism of the glucose (i.e., transport is not rate-limiting) (33–36). Thus, glucose can reach high concentrations intracellularly, especially during periods of hyperglycemia.

Transport of glucose and several other hexoses across plasma membranes is mediated mainly by a gene family of Na<sup>+</sup>-independent glucose transporters; of this family, there are at least 7 different forms (37). GLUT1 is a widely expressed isoform that is also expressed at sites of barrier function, such as the blood-retinal and blood-brain barriers (38). Glucose reaches individual cells of the retina and brain normally via GLUT1-mediated transfer of the sugar across the microvascular endothelial cells from the blood or across the RPE. Transport of glucose into some nonvascular cells of the retina and brain is also mediated by GLUT1, with the GLUT1 transporter having been identified immunohistochemically in glial

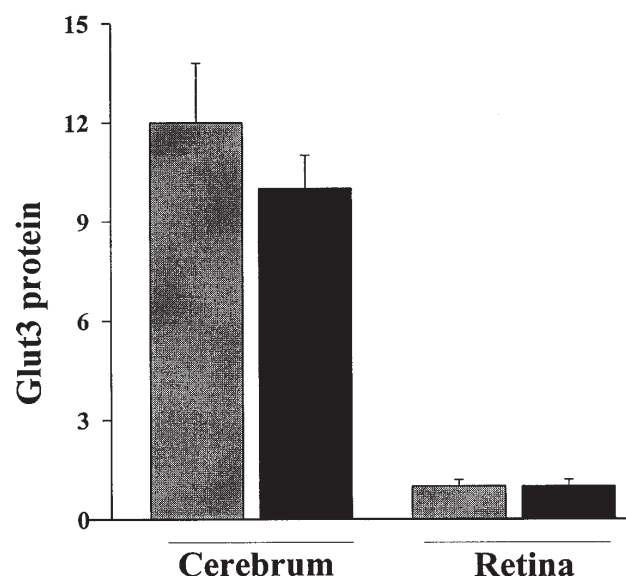


FIG. 5. GLUT3 expression in retina and cerebral cortex is unaffected by diabetes. The duration of diabetes is 2 weeks. The y-axis is *n*-fold the value of nondiabetic retina. Data are means  $\pm$  SE ( $n = 8$  per group). □, Nondiabetic rats; ■, diabetic rats.

cells, ganglion cells, photoreceptors, and pigment epithelial cells (39–41). Low levels of GLUT3 also are detectable within retina. GLUT3 is highly expressed in cerebral neurons (16,38,42–44), suggesting that this isoform mediates glucose transport into neuronal cells.

Retina and cerebral cortex are embryologically similar, yet we and others (9–11) have shown them to differ in their susceptibility to develop microvascular lesions in diabetes. Cerebral cortical vessels of dogs with either diabetes or experimental galactosemia of a 5-year duration were found to possess none of the microaneurysms, acellular capillaries, and pericyte ghosts that occurred in retinal vessels of the very same animals (11). Differences in tissue architecture or in activity of particular enzymes might account for the tissue difference in susceptibility to developing diabetic microvascular disease, but differences in the amount of glucose transport into the tissues might also be a factor.

There have been several reports on the effects of diabetes on GLUT1 expression in cerebral cortex and cerebral microvessels, but the results of these reports remain controversial. Investigators have reported a decrease (12,13,15,16) or no changes (14,45) in GLUT1 expression in cerebral microvessels of diabetic rats. Likewise, the density of cytochalasin B-binding sites, which reflect GLUT1 concentration in the vascular endothelium (because GLUT1 is the major glucose transporter present in those cells), has yielded contradictory results; glucose-displaceable cytochalasin B-binding has been reported to be decreased, increased, and unchanged in cerebral microvessels of diabetic rats (14). Some investigators have reported increased levels of GLUT1 mRNA in cerebral microvessels in diabetes despite normal GLUT1 protein concentrations (45), suggesting a diabetes-induced defect in translation of the transporter.

Effects of diabetes on the *in vivo* expression of GLUT1 in retina and its microvessels has been reported by only a single laboratory (17,39). These authors initially reported that diabetes did not alter the distribution of GLUT1 in the retina, but they

were unable to determine if the amount of the transporter was altered (39). The same investigators later quantitated the effect of diabetes on GLUT1 expression in retinal microvessels, although the analysis was based on only 3 patients (17). In those 3 patients, diabetes was associated with an increase or no change in the expression of GLUT1 in the retinal endothelium. Other studies from the same laboratory (using the same immunochemical methods) reported that diabetes decreased expression of GLUT1 in endothelial cells of the cerebral cortex (13), thus offering a possible mechanism for the different susceptibilities of retinal and cerebral microvessels to hyperglycemia-induced microvascular disease.

Our studies, however, do not support the aforementioned conclusions. Our findings, based on Western blots of whole tissue and isolated microvessels from each tissue, indicate that diabetes for up to a 2-month duration decreased expression of GLUT1 in neural retina, whereas expression of the transporter in cerebral cortex remained largely unaffected. The microvascular density at 2 months of diabetes was not abnormal; thus, the observed decrease in GLUT1 content of the retina was not secondary to diabetes-induced changes in vascular density. The different conclusions between our study and that of Kumagai et al. (17) might be due to differences in species, durations of diabetes, and methods of GLUT1 quantitation. Quantitation of GLUT1 in the studies of Kumagai et al. (17) and Pardridge et al. (13) was very precise, because it involved ultrastructural localization and quantitation of GLUT1 sites, but their analyses included only a small number of capillaries from each tissue. In contrast, our studies involved essentially the entire vasculature of the retina, but the method we used lacked the ability to determine the cellular and subcellular distribution of the transporters. More work must be accomplished to resolve the different conclusions of the studies and to determine their significance.

The RPE constitutes the second site of the blood retinal barrier. It is estimated that a significant amount of all glucose enters the retina via the RPE, and these estimates are consistent with the presence of appreciable amounts of GLUT1 in that cell type. In our studies, diabetes of a 2-month duration had no effect on the expression of GLUT1 in the RPE, despite significantly inhibiting expression of the transporter in the retinal vasculature. Regulation of GLUT1 expression apparently is under different control in different cell types. In diabetes, the combination of increased systemic glucose concentration and unchanged expression of GLUT1 in RPE will likely result in a considerable increase in total glucose transport into the retina across the RPE. Moreover, a greater fraction of the glucose entering the retina in diabetes is likely to occur via the RPE due to the decrease in GLUT1 expression in retinal microvessels.

The present comparisons suggest that the retina and its vasculature express more GLUT1 than the cerebrum and its vasculature. Thus, even though GLUT1 expression was downregulated in retinal vessels in diabetes, it is probable that glucose transport across retinal vessels remained at least as great as that across cerebral vessels of diabetic rats. Rates of glucose transport and metabolism in diabetes need to be compared in retina and cerebral cortex to more fully understand the difference in susceptibility of retina and cerebrum to diabetic microvascular disease. In diabetes, downregulation of GLUT1 in retinal microvessels likely minimizes excess transport of glucose across the vasculature, but

unchanged expression of the transporter in RPE probably diminishes that effect by allowing a greater fraction of glucose entry into the retina across the RPE. Ultimately, the downregulation of GLUT1 in retinal vessels in diabetes is not sufficient to prevent diabetes-induced retinopathy.

#### ACKNOWLEDGMENTS

This study was supported in part by support from the National Institutes of Health (grant nos. EY003000 [to T.S.K.] and DK45945 [to F.I.-B.]) and the Medical Research Service of the Department of Veteran Affairs (T.S.K.). G.A.B. was supported by a grant from the Government of Egypt, and J.T. is a recipient of a fellowship from the Lions Club International Sightfirst program, which is administered by the American Diabetes Association.

Support from the Diabetes Association of Greater Cleveland is also acknowledged.

#### REFERENCES

- Engerman RL, Kern TS: Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 33:97-100, 1984
- Kador PF, Akagi Y, Takahashi Y, Ikebe H, Wyman M, Kinoshita JH: Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol* 108:1301-1309, 1990
- Robison WG Jr, Tillis TN, Laver N, Kinoshita JH: Diabetes-related histopathologies of the rat retina prevented with an aldose reductase inhibitor. *Exp Eye Res* 50:355-366, 1990
- Kern TS, Engerman RL: Comparison of retinal lesions in alloxan-diabetic rats and galactose-fed rats. *Curr Eye Res* 13:863-867, 1994
- Kern TS, Engerman RL: A mouse model of diabetic retinopathy. *Arch Ophthalmol* 114:986-990, 1996
- Engerman RL, Bloodworth JMB Jr, Nelson S: Relationship of microvascular disease in diabetes to metabolic control. *Diabetes* 26:760-769, 1977
- Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
- U.K. Prospective Diabetes Study: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet* 352:837-853, 1998
- de Oliveira F: Pericytes in diabetic retinopathy. *Br J Ophthalmol* 50:134-143, 1966
- Addison DJ, Garner A, Ashton N: Degeneration of intramural pericytes in diabetic retinopathy. *Br Med J* 1:264-266, 1970
- Kern TS, Engerman RL: Capillary lesions develop in retina rather than cerebral cortex in diabetes and experimental galactosemia. *Arch Ophthalmol* 114:306-310, 1996
- Choi TB, Boado RJ, Pardridge WM: Blood-brain barrier glucose transporter mRNA is increased in experimental diabetes mellitus. *Biochem Biophys Res Comm* 164:375-380, 1989
- Pardridge WM, Triguero D, Farrell CR: Downregulation of blood-brain barrier glucose transporter in experimental diabetes. *Diabetes* 39:1040-1044, 1990
- Pelligrino DA, LaManna JC, Duckrow RB, Bryan RM Jr, Harik SI: Hyperglycemia and blood-brain barrier glucose transport. *J Cereb Blood Flow Metab* 12:887-899, 1992
- Taarnhoj J, Alm A: The effect of diabetes on transport through the blood-retinal and blood-brain barriers in rats. *Graefes Arch Clin Exp Ophthalmol* 229:291-293, 1991
- Kainulainen H, Schurmann A, Vilja P, Joost HG: In vivo glucose uptake and glucose transporter proteins GLUT1 and GLUT3 in brain tissue from streptozotocin-diabetic rats. *Acta Physiol Scand* 149:221-225, 1993
- Kumagai AK, Vineros SA, Pardridge WM: Pathological upregulation of inner blood-retinal barrier GLUT-1 glucose transporter expression in diabetes mellitus. *Brain Res* 706:313-317, 1996
- Morgan CR, Lazarow A: Immunoassay of insulin: two-antibody system: plasma insulin levels of normal, sub-diabetic and diabetic rats. *Diabetes* 12:115-126, 1963
- Cingle KA, Kalski RS, Bruner WE, O'Brien CM, Erhard P, Wyszynski RE: Age-related changes of glycosidases in human retinal pigment epithelium. *Curr Eye Res* 15:433-438, 1996
- Shi J, Simpkins JW: 17  $\beta$ -Estradiol modulation of glucose transporter 1 expression in blood-brain barrier. *Am J Physiol* 272:E1016-E1022, 1997

21. Sussman I, Carson MP, McCall AL, Schultz V, Ruderman NB, Tornheim K: Energy state of bovine cerebral microvessels: comparison of isolated methods. *Microvasc Res* 35:167-178, 1988
22. Kowluru RA, Jirousek MR, Stramm LE, Farid NA, Engerman RL, Kern TS: Abnormalities of retinal metabolism in diabetes or experimental galactosemia. V. Relationship between protein kinase C and ATPases. *Diabetes* 47:464-469, 1998
23. Elias H, Hennig A, Schwartz DE: Stereology: applications to biomedical research. *Physiol Rev* 51:158-200, 1971
24. Engerman RL, Kern TS: Hyperglycemia as a cause of diabetic retinopathy. *Metabolism* 35 (Suppl. 1):20-23, 1986
25. Hammes H-P, Martin S, Federlin K, Geisen K, Brownlee M: Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci U S A* 88:11555-11558, 1991
26. Engerman RL, Kern TS: Aldose reductase inhibition fails to prevent retinopathy in diabetic and galactosemic dogs. *Diabetes* 42:820-825, 1993
27. Kern TS, Kowluru R, Engerman RL: Abnormalities of retinal metabolism in diabetes or galactosemia: ATPases and glutathione. *Invest Ophthalmol Vis Sci* 35:2962-2967, 1994
28. Kowluru R, Kern TS, Engerman RL: Abnormalities of retinal metabolism in diabetes or galactosemia. II. Comparison of  $\gamma$ -glutamyl transpeptidase in retina and cerebral cortex, and effects of antioxidant therapy. *Curr Eye Res* 13:891-896, 1994
29. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL: Characterization of the mechanism for the chronic activation of DAG-PKC pathway in diabetes and hypergalactosemia. *Diabetes* 43:1122-1129, 1994
30. Kowluru RA, Kern TS, Engerman RL, Armstrong D: Abnormalities of retinal metabolism in diabetes or experimental galactosemia. III. Effects of antioxidants. *Diabetes* 45:1233-1237, 1996
31. Kowluru RA, Kern TS, Engerman RL: Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. *Free Radic Biol Med* 22:587-592, 1996
32. Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, King GL: Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest* 98:2018-2026, 1996
33. Goldstein GW, Csejtey J, Diamond I: Carrier-mediated glucose transport in capillaries isolated from rat brain. *J Neurochem* 28:725-728, 1977
34. Betz AL, Csejtey J, Goldstein GW: Hexose transport and phosphorylation by capillaries isolated from rat brain. *Am J Physiol* 236:C96-C102, 1979
35. Betz AL, Goldstein GW: Transport of hexoses, potassium, and neutral amino acids into capillaries isolated from bovine retina. *Exp Eye Res* 30:593-605, 1980
36. Betz AL, Bowman PD, Goldstein GW: Hexose transport in microvascular endothelial cells cultured from bovine retina. *Exp Eye Res* 36:269-277, 1983
37. Mueckler M: Family of glucose-transporter genes: implication for glucose homeostasis and diabetes. *Diabetes* 36:6-11, 1990
38. Vannucci SJ, Maher F, Simpson IA: Glucose transporter proteins in brain: delivery of glucose to neurons and glia. *Glia* 21:2-21, 1997
39. Kumagai AK, Glasgow BJ, Partridge WM: GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. *Invest Ophthalmol Vis Sci* 35:2887-2894, 1994
40. Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H: Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in cells of the blood-retinal barrier in the rat. *Invest Ophthalmol Vis Sci* 33:377-383, 1992
41. Vilchis C, Salceda R: Characterization of [ $^3$ H]deoxy-D-glucose uptake in retina and retinal pigment epithelium or normal and diabetic rats. *Neurochem Int* 28:213-219, 1996
42. Badr GA, Zhang J-Z, Tang J, Kern TS, Ismail-Beigi F: Glut1 and Glut3 expression, but not capillary density, is increased by cobalt chloride in rat cerebrum and retina. *Brain Res Mol Brain Res* 64:24-33, 1999
43. Knott RM, Robertson M, Muckersie E, Forrester JV: Regulation of glucose transporters (GLUT-1 and GLUT-3) in human retinal endothelial cells. *Biochem J* 318:313-317, 1996
44. Vannucci SJ, Seaman LB, Vannucci RC: Effects of hypoxia-ischemia on GLUT1 and GLUT3 glucose transporters in immature rat brain. *J Cereb Blood Flow Metab* 16:77-81, 1996
45. Vannucci SJ, Gibbs EM, Simpson IA: Glucose utilization and glucose transporter proteins GLUT-1 and GLUT-3 in brains of diabetic (*db/db*) mice. *Am J Physiol* 272:E267-E274, 1997