Glial Reactivity and Impaired Glutamate Metabolism in Short-Term Experimental Diabetic Retinopathy

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The early pathophysiology of diabetic retinopathy and the involvement of neural and vascular malfunction are poorly understood. Glial cells provide structural and metabolic support for retinal neurons and blood vessels, and the cells become reactive in certain injury states. We therefore used the streptozotocin rat model of short-term diabetic retinopathy to study glial reactivity and other glial functions in the retina in the first months after onset of diabetes. With a two-site enzyme-linked immunosorbent assay, we measured the expression of the intermediate filament glial fibrillary acidic protein (GFAP). After 1 month, GFAP was largely unchanged, but within 3 months of the beginning of diabetes, it was markedly induced, by fivefold (P < 0.04). Immunohistochemical staining showed that the GFAP induction occurred both in astrocytes and in Müller cells. Consistent with a glial cell malfunction, the ability of retinas to convert glutamate into glutamine, assayed chromatographically with an isotopic method, was reduced in diabetic rats to 65% of controls (P <0.01). Furthermore, retinal glutamate, as determined by luminometry, increased by 1.6-fold (P < 0.04) after 3 months of diabetes. Taken together, these findings indicate that glial reactivity and altered glial glutamate metabolism are early pathogenic events that may lead to elevated retinal glutamate during diabetes. These data are the first demonstration of a specific defect in glial cell metabolism in the retina during diabetes. These findings suggest a novel understanding of the mechanism of neural degeneration in the retina during diabetes, involving early and possibly persistent glutamate excitotoxicity. ***Diabetes*** **47:815–820, 1998**

Treatment of diabetic retinopathy is hampered by the lack of understanding of early pathogenic processes, particularly as they relate to neural cells of the retina. Vascular changes set in after 10–15 years of diabetes in humans (1), and after more than a year in rat models of diabetes (2). However, little is understood about changes to neurons and glia during the first years of diabetes in humans and the first months in animals, a time when the vasculature appears normal by histological criteria.

Several observations indicate that the degenerative processes begin in the neural retina shortly after the onset of diabetes and precede the characteristic vascular changes that prompt the diagnosis of retinopathy. Loss of color sensitivity (3,4) and contrast sensitivity (5,6) are early signs of neural retinal dysfunction that occur after just 2 years of diabetes. Furthermore, the oscillatory potentials on the b-wave of electroretinograms become altered after only a few years in human diabetic patients (7). In rats, similar neurophysiological changes are observed within 2 weeks of diabetes onset (8). These findings suggest that diabetic retinopathy is a primary neurosensory disorder, as initially proposed by Bresnick (9). It is thus important to characterize early pathological processes in the neural retina in diabetes prior to the onset of vascular pathology and visual loss.

Glial cells play a central role in the homeostatic regulation of the retina (10). Normal neuronal and vascular function relies on interactions with glia. Glial cells are implicated in the maintenance of the blood-retinal barrier at the endothelial lining of retinal microvessels (11), as has also been shown for the blood-brain barrier (12). The extracellular ionic environment is optimized by glia for proper electrophysiological function of neurons. Also, glia maintain low synaptic levels of neurotransmitters. The major excitatory neurotransmitter in the retina is glutamate, an amino acid that is toxic to retinal neurons when present in high abundance (13). Thus, the regulation of retinal glutamate by glia (14) is essential for normal vision. The proliferation of glial cells in the formation of preretinal membranes has been reported (15), but glial involvement in early pathological events in diabetic retinopathy has not been investigated. Given their important role in normal vision, it is critical to understand the function of retinal glia during diabetes.

Here we report that metabolic changes occur in the retina soon after the onset of experimental diabetes in streptozotocin (STZ) diabetic rats. By 3 months of diabetes, glial fibrillary acidic protein (GFAP) immunoreactivity (IR) is significantly increased. Consistent with altered glial function, glutamate handling and tissue levels of glutamate in the retina change. These findings therefore suggest that neurodegenerative events may occur early during diabetes and precede proliferative and hemorrhagic vascular changes in the retina during diabetes. Parts of this work have been presented in preliminary form (16).

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ELISA, enzyme-linked immunosorbent assay; FNM, flavin mononucleotide; GFAP, glial fibrillary acidic protein; IR, immunoreactivity; PBS, phosphate-buffered saline; PBST, PBS/0.1% Triton X-100; PCA, perchloric acid; STZ, streptozotocin.

RESEARCH DESIGN AND METHODS

Diabetic animals. Eight-week-old Sprague Dawley rats (~200 g) obtained from Charles River (Wilmington, MA) were made diabetic by tail-vein injection of STZ (Sigma, St. Louis, MO; 65 mg/kg body weight in 0.1 mol/l citrate-buffered saline, pH 4.5). Age-matched control rats were not injected. Animals were judged diabetic within 2 days if blood glucose rose to >250 mg/dl. After 1 month, diabetic rats were maintained with weekly injections of 2 U insulin (Ultralente, Humulin). After 1 or 3 months, retinas were dissected and either frozen immediately for biochemistry or histochemistry or incubated for assays of glutamate metabolism. Zucker rats were bred in the animal facility at the Pennsylvania State University College of Medicine. Zucker rats were housed for ~9 months and determined to be either diabetic or nondiabetic based on weight gain and tail length. All rats received food and water ad libitum and were maintained on a 12-h light/12-h dark cycle. Animals were cared for in accordance with the ARVO Resolution on the Care and Use of Laboratory Animals.

GFAP enzyme-linked immunosorbent assay (ELISA). Relative GFAP IR was determined by a minor modification (S. Levison, unpublished data) of published methods (17). Dissected retinas were sonicated in extraction buffer (10 mmol Tris, 0.1% Triton X-100, 1 mmol dithiothreitol, 5 mmol $MgCl_2$, 5 mmol EGTA, 150 mmol NaCl, 0.2 mmol phenylmethylsulfonyl fluoride) plus 6 mmol/l urea. Homogenates were centrifuged at 4°C (10 min, 14,000 rpm). The supernatants were then diluted 1:10 in extraction buffer without urea, and protein concentrations were determined by the D_C protein assay (BioRad, Hercules, CA). Ninety-six-well microtiter plates (Falcon) were coated with 100 µl of rabbit anti-GFAP (DAKO, 1/400) per well and incubated at 4°C for 5–6 h. After incubation, the wells were washed 6 times with phosphate-buffered saline (PBS; 200 µl/well). Nonspecific binding was blocked with ELISA-blotto (PBS, 0.5% nonfat dry milk) at room temperature for 1 h. The samples and standards were diluted to 100 µg/ml in PBS/0.1% Triton X-100 (PBST). Triplicate samples of 10 µg of protein were added per well and incubated overnight at 4°C. Plates were washed in PBST and then incubated for 1 h at 37°C in mouse anti-GFAP (Boehringer Mannheim, Indianapolis, IN; 1:500 in ELISA-blotto + 0.5% Triton X-100). After another wash in PBST, well contents were incubated in alkaline phosphatase conjugated goat anti-mouse (ICN Pharmaceuticals, Costa Mesa, CA; 1:1,000 in ELISA-blotto + 0.5% Triton X-100) and then reacted with *P*-nitrophenyl phosphate (Southern Biotechnology, Birmingham, AL). Optical density was read at 405 nm in an ELISA plate reader (Molecular Probes, Sunnyvale, CA).

Immunohistochemistry. Immunohistochemistry was carried out by a combination of methods previously described (18–20). Ten-micrometer cryostat sections were fixed in 2% paraformaldehyde for 10 min and then washed in PBS. Nonspecific binding was blocked with PBST and 10% goat serum. All subsequent antibody incubations were carried out in this buffer. Between incubations, sections were washed in PBST. After the block step, sections were incubated at 4°C overnight in anti-GFAP monoclonal antibody (Boehringer Mannheim; 1:400), followed by biotinylated horse anti-mouse (Pierce, Rockford, IL; 1:200) and then streptavidin-fluorescein isothiocyanate (Jackson, West Grove, PA; 1:400). Secondary and tertiary reagent incubations were at room temperature for 1 h each, separated by washes for at least 1 h, with at least three changes. Specimens were mounted in Aquamount (Polysciences, Warrington, PA) and photographed using a CCD-based image acquisition system on an Olympus B-Max50 fluorescence microscope.

Immunoblotting. Immunoblotting for GFAP was done by standard methods, as previously published (18). Retinas were homogenized and diluted in sample buffer before loading 100–200 µg protein per lane. After electrophoresis, proteins were transferred to nitrocellulose by wet electroblotting. All subsequent block and antibody incubations were done in western-blotto (3% nonfat dried milk, 0.1% Tween-20, 1% goat serum), and all washes were in Tris-buffered saline supplemented with 0.5% Tween-20. Membranes were blocked in western-blotto, incubated overnight at room temperature in mouse anti-GFAP (Boehringer Mannheim; 1:400), washed, incubated 1 h in ^{125}I-sheep anti-mouse (New England Nuclear, Boston, MA; 0.2 µCi/ml), and washed. Images were acquired by phosphoimaging (Phosphoimager SI; Molecular Dynamics).

Glutamate-to-glutamine conversion. Retinas were removed by dissection in PBS. One retina from each animal was incubated separately for 30 min in 5 ml Ringer's buffer containing 2 mmol glutamine and 50 µmol [^{14}C]glutamate (specific activity 2,000 cpm/nmol) at 37°C. Retinas were then removed to 3% perchloric acid (PCA). After sonication and centrifugation, the pellets were assayed for protein (BCA; Pierce) and supernatants neutralized. Incubation buffer was recovered and immediately acidified (3% PCA). Samples were neutralized to pH 6.5 with KOH and held at –20°C until further processing. They were then applied to 2-ml acetate columns and fractionated on an acetic acid gradient by ion exchange chromatography to separate and measure radiolabeled glutamate and glutamine, as described (21). Neutral amino acids, including glutamine, appeared in the flow-through fraction. This fraction was incubated in asparaginase (Boehringer Mannheim) for 2 h at room temperature. This step quantitatively converted glutamine to glutamate, which could then be refractionated. The glutamate fractions from each retina were counted by scintillation counting and normalized for retinal protein. Data were expressed as nanomoles of glutamine synthesized per milligram of retina.

Glutamate determinations. Glutamate mass was determined by luminometry using a minor modification of published methods (21). Retinas were dissected from normal and diabetic rats and dissociated as described above for ELISA. Protein concentration was determined by standard procedure (BioRad). Samples were diluted 10-fold in reaction buffer (100 mmol Tris base, 400 mmol hydrazine hydrate, 10 mmol $MgCl_2$, 5 mmol EDTA, pH 8.5) supplemented with 1.6 mg/ml NAD (Sigma) and 0.6 U/ml glutamate dehydrogenase (Boehringer Mannheim). Samples were incubated for 30 min at room temperature, converting glutamate quantitatively to α-ketoglutarate and NADH. The reaction was diluted 200-fold with 50 mmol triethanolamine. To measure NADH, the samples were then mixed 1:1 with luminometer buffer (25 mmol potassium phosphate, 100 µmol dithiothreitol, 5 mg/l $FMNH_2$-dependent luciferase, 80 U/l flavin mononucleotide (FMN) reductase, 2.5 µmol FMN, 50 mg/l Triton, 24 µmol myristic aldehyde) for luminometry. Luminosity was measured in a luminometer (AutoLumat LB953; Berthold, Wellesley, MA) and compared with a standard glutamate concentration curve.

Statistical analysis. Data were analyzed for statistical significance by the Mann-Whitney rank-sum test or the Student's t test, as indicated. In all cases, $P < 0.05$ was considered statistically significant.

RESULTS

Increased GFAP indicates altered glial function in diabetic rat retina. Glial reactivity is a frequent nervous system response to injury (22). Activation of glia alters their ability to regulate nervous system homeostasis, so we examined the possibility that glia become reactive in diabetic retinopathy. A common marker for reactive gliosis is the well-described increase in the expression of the intermediate filament protein GFAP (23). We measured the expression of GFAP using a two-site ELISA. Twenty Sprague Dawley rats were made diabetic and housed for either 1 month or 3 months. Ten age-matched, control animals were not made diabetic. At the end of the specified duration of diabetes, retinas were dissected and either homogenized for ELISA or sectioned for immunohistochemistry (see METHODS).

ELISA measurement of retinal GFAP after 1 month showed no change in the overall amount of IR (Fig. 1). However, as seen in Fig. 1, GFAP IR in 3-month diabetic rats is

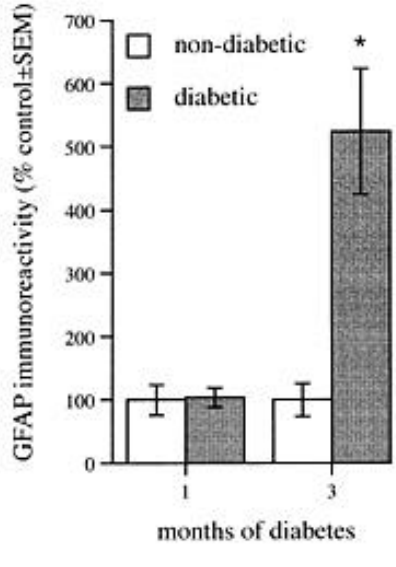

FIG. 1. Increased GFAP indicates glial reactivity in the retina in short-term diabetes. Retinas were dissected from control and diabetic rats after 1 or 3 months and homogenized for assay by two-site ELISA quantified by alkaline phosphate conversion of *P*-nitrophenyl phosphate. A fivefold increase in GFAP expression was observed after 3 months of diabetes (*$P < 0.04$, Mann-Whitney).

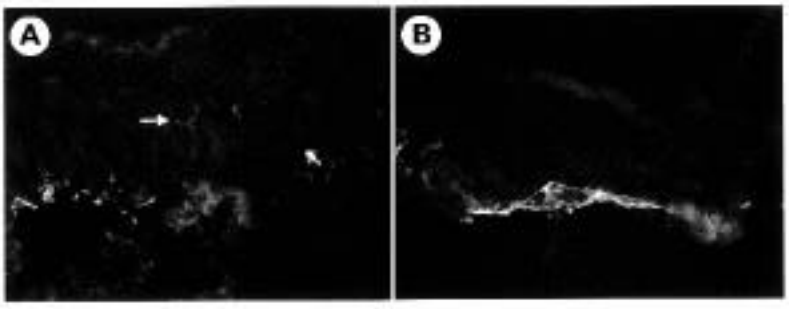

FIG. 2. Occasional Müller cells of the retina have increased GFAP IR at 1 month of diabetes. Retinas from diabetic (*A*) or nondiabetic (*B*) rats were dissected, frozen, and cryosectioned for immunofluorescent staining with anti-GFAP antibodies. In some sections, isolated Müller cells stain with anti-GFAP (arrow). No Müller cell GFAP IR was observed in any nondiabetic animals using this method. Retinal astrocytes in the ganglion cell layer (bottom) are immunoreactive in both diabetic and nondiabetic animals.

significantly increased, by fivefold ($P < 0.04$, Mann-Whitney) compared with nondiabetic controls. These data indicate that Müller cells and/or astrocytes become reactive within the first few months of diabetes.

To investigate which glial types become reactive in diabetes, we studied the distribution of GFAP in the retina. Six diabetic and six control retinas were prepared for indirect immunofluorescence as described in METHODS. Immunohistochemical staining of retinas after 1 month of diabetes with antibodies directed to GFAP showed that rare GFAP immunoreactive cells with morphology resembling Müller glia were sparsely distributed (Fig. 2) in the retina. These immunoreactive cells were never observed in nondiabetic animals, indicating that at 1 month of diabetes a small number of glial cells in the retina become reactive. We also examined the distribution of GFAP IR in rat retinas after 3 months of diabetes. Retinal GFAP IR accumulates in both Müller glia and astrocytes during diabetes, but not in normal animals. Astrocytes of the ganglion cell layer show more intense staining in the retinas of diabetic rats. Staining of Müller cells was evident in all but one diabetic animal at 3 months. Of those animals with Müller cell GFAP IR, most exhibited staining in both the inner and outer layers of the retina (Fig. 3*A*). In all animals, Müller cell compartments in the inner half of the retina were consistently stained (Fig. 3*C*). No Müller cell GFAP staining was observed in control retinas by this method.

To determine if the increased GFAP IR observed by immunohistochemistry and ELISA is due to an increase in GFAP protein, we also ran immunoblots. Retinal homogenates from three control and three diabetic rats were loaded at both 200 and 100 µg per lane, electrophoresed, electroblotted, and probed with anti-GFAP antibodies. Figure 4 (arrow) shows that GFAP, migrating at a relative mobility of ~50 kDa, markedly increases in the retinas from diabetic (4–6) compared with control rats (1–3). Omission of the primary antibody results in staining that is not distinguishable from background (not shown). The 100-µg loadings of diabetes samples stain 2–3 times more intensely than the 200-µg loadings of control samples, consistent with the fivefold increase seen by ELISA. GFAP is a phosphoprotein, and phosphorylation regulates GFAP depolymerization (24). We have observed an additional band at a lower molecular weight in some samples (Fig. 4, arrowhead). The possibility that diabetes alters the phosphorylation state of GFAP must be investigated by other methods. Our immunoblots showing increased GFAP in retinas of short-term diabetic rats, together with the ELISA and immunofluorescence data, demonstrate that glial reactivity is an early pathogenic event that begins within the first 3 months of experimental diabetes in the rat.

Decreased metabolism of glutamate in diabetic rat retinas indicates glial malfunction. Glial reactivity is an indication of altered glial function. One of the important glial

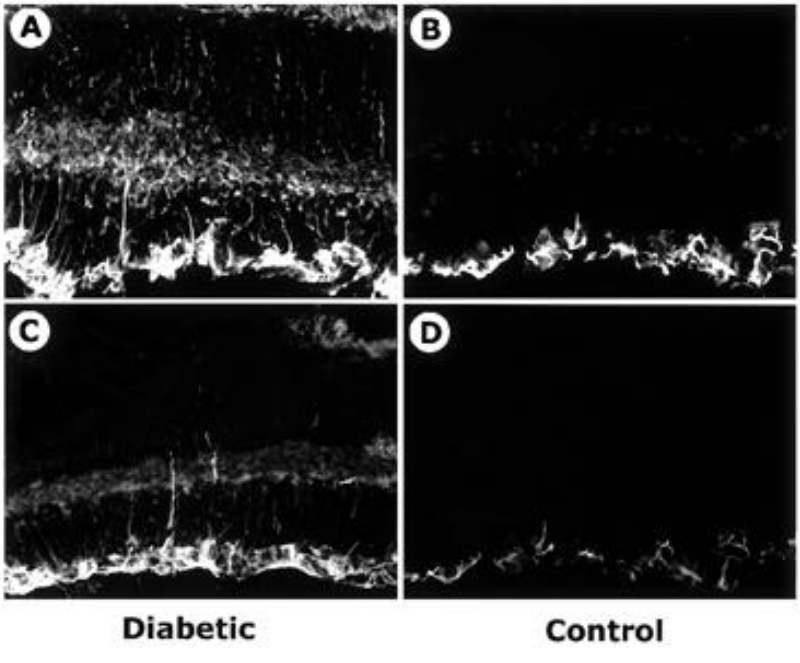

FIG. 3. Glial reactivity in the retina during diabetes manifests as increased GFAP IR in Müller cells and astrocytes. Retinas from diabetic (*A, C*) and nondiabetic (*B, D*) rats were prepared for GFAP immunofluorescence and oriented as in Fig. 2. Note that in some animals (e.g., *A*) Müller cell GFAP IR spans the entire depth of the neural retina, whereas in other animals (e.g., *B*) Müller cell GFAP IR is restricted to the inner layers. Also, there is considerable variability in the intensity of GFAP IR between Müller cells within any given retina. All 3-month diabetic animals demonstrated notably increased Müller cell GFAP IR compared to 1-month diabetic animals (see Fig. 2).

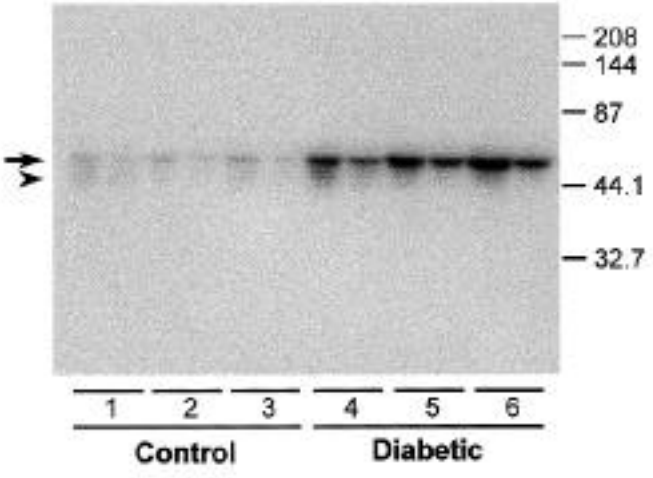

FIG. 4. Immunoblotting confirms that increased GFAP IR in diabetes is due to increased GFAP. Two hundred or 100 µg of retinal protein was loaded onto left and right lanes, respectively, for control (*lanes 1–3*) and diabetic (*lanes 4–6*) animals. GFAP was visualized by incubating in mouse anti-GFAP and ^{125}I-sheep anti-mouse before exposure on a phosphoimager. The major band representing GFAP migrates at a relative mobility of about 50 kDa (arrow). However, a second band appears at a lower molecular weight (~45–47 kDa) in some samples (arrowhead).

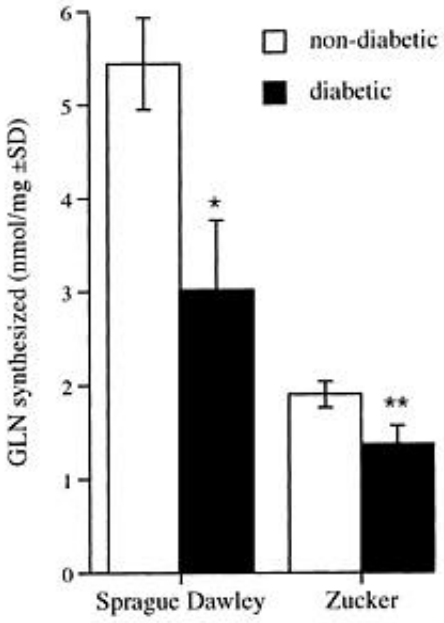

FIG. 5. Impaired glutamine synthesis in the retina during diabetes. Retinas were dissected and incubated in [^{14}C]glutamate for 30 min at 37°C before sonication and acetate chromatography to determine glutamine synthesized. Diabetes was induced in Sprague-Dawley rats by injection with STZ. Zucker rats were assessed as diabetic based on body weight and tail length. In both models, retinas synthesized significantly less glutamine (*P < 0.01, **P < 0.02, respectively, Student's t test) during diabetes.

functions in the retina is to metabolize the excitatory neurotransmitter glutamate after synaptic release (14). To determine if glial glutamate metabolism is disturbed in diabetes, we measured the ability of retina to convert glutamate into glutamine. Sprague-Dawley rats were made diabetic and maintained with minimal insulin for 9 months along with age-matched controls. Retinas were then dissected and synthesis of glutamine was measured after a 30-min incubation of [^{14}C]glutamate at 37°C (see METHODS). As depicted in Fig. 5, STZ diabetic rats converted significantly less glutamate to glutamine (P < 0.01). Furthermore, retinas of Zucker obese rats, a model for type 2 diabetes, also demonstrated a diminished capacity to metabolize glutamate (P < 0.02). These findings indicate that glutamate metabolism by retinal glia is impaired in diabetes, consistent with the accumulation of glutamate in retinas with diabetes.

Glutamate elevation in retinas of rats with short-term diabetes. Glutamate excitotoxicity has been proposed as a pathogenic mechanism for retinal neurons (13,25). Consequently, we determined whether accumulation of retinal glutamate accompanies its altered metabolism. We measured glutamate luminometrically (see METHODS) in homogenates from retinas of rats after 3 months of STZ-induced diabetes compared with age-matched controls. Normal retinas contain 35.2 ± 6.8 nmol glutamate per mg protein (Fig. 6). Three months of diabetes causes a 1.6-fold glutamate increase to 56.9 ± 7.0 nmol/mg (P < 0.04). Therefore, within the first months of experimental diabetes, glutamate accumulates in the retina, where it may act as a toxin for ganglion cells and other retinal neurons.

DISCUSSION

In the experiments reported here, we have investigated the involvement of glial function in early changes of the retina during diabetes. We found that both astrocytes and Müller cells of the retina become reactive within 3 months of diabetes in the rat. Glial reactivity is observable as increased GFAP IR in the ganglion cell layer and also in the Müller cell fibers that run perpendicular to the plane of the retina. Most animals show an increase in Müller cell GFAP IR in the inner plexiform and inner nuclear layers, and some animals also showed GFAP IR in the outer layers of the retina. Consistent with a glial reaction to injury, we also found decreased metabolism of glutamate in the retina as a decrease in the conversion of glutamate to glutamine to 65% of control. Because one of the possible consequences of decreased glutamate metabolism in

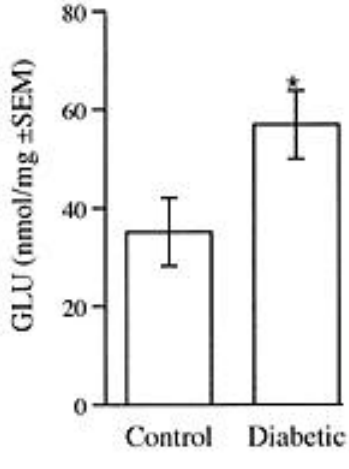

FIG. 6. Glutamate accumulation in retinas of rats with short-term diabetes. Retinas from nondiabetic and 3-month diabetic rats were sonicated, and glutamate was measured luminometrically. Diabetes at this time point induces a 1.6-fold accumulation of glutamate compared to nondiabetic control levels (*P < 0.04).

the retina may be increased extracellular accumulation of glutamate, we also studied the glutamate levels in the retina and found significantly increased glutamate in retinas of diabetic as compared with control rats.

Glial reactivity is widely regarded as an alteration in glial function during injury and has been studied in the brain and spinal cord (22), as well as in the retina. In the retina, it can be caused by a variety of insults and involves both Müller and astroglial cells (23,26). Glial responses in nervous-system injury include expression of growth factors and modulation of enzymes such as glutamine synthetase (22,27). The most constant manifestation of reactivity is the increase in IR for the intermediate filament protein GFAP. The significance of GFAP upregulation is as yet unclear. Indeed, it is not possible to determine from our data if all of the increase in GFAP IR at 3 months of diabetes is due to an overall increase in GFAP expression or whether the increased GFAP IR is due to epitope unmasking. The latter explanation is unlikely because we show here that the increase is observed by immunoblotting, where the samples are boiled in SDS before electrophoresis. Also, various investigators have used a variety of immune reagents to describe increased GFAP. Furthermore, increased GFAP expression was also demonstrated by immunoblotting of human retinas after the onset of proliferative diabetic retinopathy (28,29). Therefore, our findings of increased GFAP IR in early experimental diabetic retinopathy are consistent with the hypothesis that glial reactivity is one of the earliest pathological events in the retina during diabetes. Our results indicate that retinal reactivity in the STZ rat begins around 1 month of diabetes and escalates during the 2nd and 3rd month. This is consistent with the findings of Hammes et al. (30), who observed Müller cell GFAP staining at 15 weeks. We are currently investigating the acceleration of glial reactivity between 1 and 3 months of diabetes.

We investigated the concentration of glutamate in the retina and found it to be increased after 3 months of experimental diabetes. A recent report observed elevated glutamate levels in the vitreous humor of humans with proliferative diabetic retinopathy (31), indicating that glutamate elevation is a persistent phenomenon in the retinas of individuals with diabetes. In that study, no other amino acid was elevated, with the exception of γ-aminobutyric acid. Together with our data, these findings suggest a specific defect in glutamate metabolism rather than a general alteration in all amino acids. However, in another study on experimental diabetes, elevated glutamate was not observed in early diabetes (32). The reasons for this discrepancy are currently unclear; however, some methodological differences, such as animal strains and analytical methods, must be investigated.

A number of laboratories have investigated the possibility that glutamate excitotoxicity can kill neurons in the retina (33,34). Glutamate excitotoxicity has been proposed as a mechanism of neurodegeneration in glaucoma (35), and in glaucoma patients, intravitreal glutamate is elevated to an extent similar to the increase we have observed. Direct intravitreal injection that increases glutamate by two- to threefold causes neurodegeneration of ganglion cells that can be decreased with *N*-methyl-D-aspartate (NMDA) receptor blocker (25). Also, cultured retinal neurons are susceptible to excitatory amino acid toxicity (13), indicating that an active cell death process, such as apoptosis, causes the cells to die. We are currently investigating the possibility that neural cells in the retina die by apoptosis early in diabetic retinopathy (A.J.B., E.L., D. Antonetti, A. Buchanan, T. Gardner, unpublished observations).

Our observation of glial reactivity and altered glutamate metabolism opens the interesting possibility that other glial functions, such as maintenance of the blood-retinal barrier (11,36), may be disturbed in diabetic retinopathy. Vascular permeability is one of the hallmarks of diabetic retinopathy (2), and glial malfunction could explain a breakdown in blood-retinal barrier. Indeed, blood-retinal barrier breakdown is one of the early events in human and experimental diabetic retinopathy (37). We have recently observed decreased tight junction protein expression in rats with short-term diabetes (D. Antonetti, T. Gardner, S. Khin, A.J.B., E.L., unpublished observations). Also, increased endothelial cell internalization of blood-borne tracer has been demonstrated in the retina after only 6 weeks of diabetes (38). One possibility is that these microvascular responses are causally related to the contemporaneous changes in glial cell function.

Increased retinal glutamate may also stimulate further physiological changes in glial cells of the retina. For instance, reactive glia commonly proliferate (39) and become gliotic. Uchihori and Puro (40) recently reported that glutamate stimulates retinal glia to proliferate by activating glial NMDA receptors, and glial cells are involved in the formation of preretinal membranes during proliferative diabetic retinopathy (15). Further studies are needed to determine the mechanistic relationships between diabetic retinopathy and glial cell mitosis.

In summary, we report here for the first time that glial function and glutamate metabolism are altered in the retina within a few months after the onset of diabetes in rats. This observation is consistent with the possibility that similar alterations occur in humans within the first years after diabetes onset but remain subclinical until degeneration proceeds to the point where irreparable vision impairment is incurred. This indicates that in humans with diabetes, retinal glutamate may focally increase to toxic levels before measurable vision impairment and before proliferative diabetic retinopathy sets in. Furthermore, increased retinal glutamate may reduce the efficacy of neurotransmission in the inner retina. Consequently, it is critical to find the cause of this early pathology in order to avert vision loss.

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