Diabetes-Induced Dysfunction of the Glutamate Transporter in Retinal Müller Cells

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PURPOSE. A decrease in the ability of Müller cells to remove glutamate from the extracellular space may play a critical role in the disruption of glutamate homeostasis that occurs in the diabetic retina. Because this amino acid is toxic to retinal neurons and is likely to exacerbate oxidative stress, elucidation of the mechanisms by which glutamate levels are elevated in diabetes may help in the understanding of the pathogenesis of diabetic retinopathy. This study tested the hypothesis that the function of the glutamate transporter in Müller cells of the diabetic retina is compromised by a mechanism involving oxidation.

METHODS. Müller cells were freshly isolated from normal rats and those made diabetic by streptozotocin injection. The activity of the Müller cell glutamate transporter, which is electrogenic, was monitored by the perforated-patch configuration of the patch-clamp technique.

RESULTS. Four weeks after the onset of hyperglycemia, a significant dysfunction of the Müller cell glutamate transporter was detected. After 13 weeks of streptozotocin-induced diabetes, the activity of this transporter was decreased by 67%. Consistent with oxidation’s causing this dysfunction, exposure to a disulfide-reducing agent rapidly restored the activity of the glutamate transporter in Müller cells of diabetic retinas.

CONCLUSIONS. Early in the course of diabetic retinopathy, the function of the glutamate transporter in Müller cells is decreased by a mechanism that is likely to involve oxidation.

Although vascular changes are the classic hallmark of diabetic retinopathy, a number of observations suggest that microangiopathy is only one aspect of a more widespread retinal dysfunction. In diabetes, induced changes in retinal neurons and glia may precede the onset of clinically evident vascular injury. For example, loss of color and contrast sensitivity and abnormalities in the electroretinogram have been documented in patients before detection of the vascular changes that are traditionally used to diagnose diabetic retinopathy.

Early neuronal and glial alterations are also evident in rats with chemically induced diabetes. These changes include decreases in components of the electroretinogram and increased apoptosis of retinal neurons. In addition, early in the course of diabetic retinopathy, Müller cells markedly upregulate their expression of glial fibrillary acidic protein, which is a nonspecific response to pathophysiological conditions. Thus, a comprehensive understanding of diabetic retinopathy requires elucidation of the mechanisms by which diabetes affects nonvascular, as well as vascular, cells of the retina.

The focus of this study was on Müller cells, which are the principal glia of the retina. A major physiological function of these cells is to regulate the ionic and molecular composition of the retinal microenvironment. An intensively studied function of Müller cells is their uptake of synaptically released glutamate, which is a neurotransmitter at more than 90% of the synapses in the retina. Consistent with Müller cells having a vital role in regulating retinal glutamate levels, reported that treatment of rats with antisense oligonucleotides directed against the Müller cell glutamate transporter caused more than a threefold increase in the vitreal concentration of glutamate. By removing extracellular glutamate, Müller cells help to terminate transmission at glutamergic synapses. Furthermore, prompt removal of this synaptically released amino acid is necessary, because prolonged activation of glutamate receptors can kill retinal neurons. Thus, mechanisms to remove glutamate from the extracellular space efficiently are necessary for the maintenance of a healthy retina.

The role of Müller cells in maintaining a low extracellular concentration of glutamate may be particularly critical in diabetes. As in the normal retina, synaptically released glutamate must be removed. In addition, neurons in the diabetic retina must also be protected from glutamate leaking into the retina at sites where the blood-retinal barrier is compromised. Because plasma contains 100 to 300 μM of this amino acid and as little as 5 μM glutamate can be lethal to neurons, a breakdown in the blood-retinal barrier, which occurs early in diabetes, could have dire consequences for retinal function and neuronal survival. Thus, the transport of glutamate into Müller cells may be essential to prevent toxic levels of this amino acid from reaching neurons located near defects in the blood-retinal barrier.

Müller cells, whose processes completely ensheath the retinal vasculature, are well positioned to remove glutamate at sites of a breakdown in the blood-retinal barrier. However, in diabetes, the ability of Müller cells to regulate the extracellular concentration of glutamate may be compromised. Support for this possibility is that levels of this amino acid are elevated in the retinas of diabetic rats. Also, the increased concentration of glutamate in the vitreous of patients with diabetic retinopathy is likely to reflect the presence of high concentrations of this amino acid in the retina. These observations suggest that the regulatory mechanisms to control glutamate may be compromised in the diabetic retina.
An essential step in the regulation of extracellular glutamate is the transport of this amino acid into Müller cells through the high-affinity γ-glutamate/L-aspartate transporter (GLAST; the human analogue is excitatory amino acid transporter EAAT-1).\(^1\)\(^\text{30}\) A potentially critical feature of GLAST is the presence of redox-sensing elements, which regulate this transporter through thiol-disulfide redox interconversion.\(^3\)\(^1\)\(^,\)\(^3\)\(^2\) Consistent with the presence of redox-sensitive sites, Trotti et al.\(^3\)\(^1\) demonstrated that chemical oxidation or reduction altered the activity of cloned GLAST molecules that had been placed in artificial liposomes. These investigators found that oxidizing agents decrease GLAST function. Subsequently, exposure to a chemical reductant restores the activity of this transporter.\(^3\)\(^1\) However, although evidence is good that cloned GLAST expressed in liposomes possesses redox sensitivity, this has not been demonstrated for native GLAST molecules located in cells, such as Müller cells, which exclusively express this type of glutamate transporter.

A central premise of this study is that the redox sites on the GLAST molecules of Müller cells render this transporter vulnerable to diabetes-induced dysfunction. This hypothesis seems reasonable, because oxidative stress occurs in the retina early in the course of diabetes.\(^2\)\(^8\)\(^,\)\(^3\)\(^5\)\(^,\)\(^3\)\(^4\) By this mechanism, glutamate homeostasis may be disrupted in the diabetic retina.

**Methods**

**Model of Diabetes**

This study conformcd to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the University of Michigan’s University Committee on the Use and Care of Animals. After an overnight fast, 5- to 6-week-old Long-Evans rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) received an intraperitoneal injection of streptozotocin (75 mg/kg) diluted in 0.8 mL of 0.03 M citrate buffer (pH 4.7). Subsequently, the animals received food and water ad libitum. The vivarium was maintained on a 12-hour alternating light-dark cycle. Three days after streptozotocin injection, diabetes was confirmed by assaying the glucose concentration (One Touch Basic; Lifescan, Milpitas, CA) in blood obtained from the tail vein. Rats with glucose levels greater than 250 mg/dL were classified as diabetic. Age-matched rats served as the control. Immediately before the harvesting of retinal Müller cells, the blood glucose level was 378 ± 6 mg/dL in the 23 diabetic rats used in this study.

**Fresh Müller Cells**

Freshly dissociated Müller cells were prepared from rats that were killed with carbon dioxide. Immediately after death, the retinas were rapidly removed and incubated in 2.5 mL Earle’s balanced salt solution (Invitrogen, San Diego, CA), which was supplemented with 0.5 mM EDTA, 20 mM glucose, 2 mM cysteine, 0.04% D.Nase, and 15 U papain (Worthington Biochemicals, Freehold, NJ), for 40 minutes at 30°C while 95% oxygen-5% CO\(_2\) was bubbled through to maintain pH and oxygenation. After transfer to a solution containing 140 mM NaCl, 3 mM KCl, 1.8 mM CaCl\(_2\), 0.8 mM MgCl\(_2\), 3 mM BaCl\(_2\), 10 mM Na-HEPES, and 5 mM glucose at pH 7.4, with the osmolarity adjusted by less than 5% to 310 mOsm/L.

With a multistage programmable puller (Sutter Instruments, San Rafael, CA), patch pipettes were pulled from glass tubing (Corning No. 7052; Gardner Glass Co., Claremont, CA) and heat polished to tip diameters of 2 to 3 μm. The pipette solution consisted of 50 mM KCl, 65 mM K$_2$SO$_4$, 6 mM MgCl$_2$, 10 mM K-HEPES, 240 μg/mL amphotericin B, and 240 μg/mL nystatin at pH 7.4, with the osmolarity adjusted to 280 mOsmol/L. The resistances of the pipettes used were approximately 5 MΩ when tested in the bathing solution.

The pipettes were mounted in the holder of a patch-clamp amplifier (model 3900; Dagan Corp., Minneapolis, MN) and sealed to the cell bodies of Müller cells. Seals generally formed over a period of 1 to 3 seconds and reached resistances of greater than 1 GΩ. As amphotericin-nystatin perforated the patch, the access resistance to the cell usually decreased to less than 20 MΩ within 10 minutes for the Müller cells analyzed. Recordings were used after the ratio of cell membrane to series resistance became greater than 10. This ratio was monitored periodically; if the ratio decreased to below 10, the analysis of the cell was terminated. Series resistance was not corrected, but the error due to the voltage decrease across the patch pipette was always less than 10% of the applied voltage. Cell membrane capacitance was estimated by using circuits of the expander module (model 3910; Dagan Corp.). Adjustment for the calculated liquid junction potential was made after data collection.

Currents were evoked by a voltage ramp protocol, which was controlled by a software program (pClamp 8 software; Axon Instruments, Inc., Foster, CA). During a voltage ramp, the applied voltage changed from negative to positive membrane potentials at the rate of 66 mV/sec. The recorded currents were filtered at 1 kHz with a four pole Bessel filter, digitally sampled at 1-ms intervals using an acquisition system (Digidata 1200B; Axon Instruments) and stored by a computer that was equipped with software for data analysis and graphics display (pClamp 8; Axon Instruments, and Origin 6.1; OriginLab Corp., Northampton, MA, respectively).

The current-voltage relationships in Figure 2 show the mean values for five successive cycles of the voltage ramp protocol. In Figures 2, 3, and 4, the inward current induced by glutamate or 1-trans-pyrrolidine-2,4-dicarboxylate (PDC) is the mean difference between the amplitude of the current at −120 mV during the 30-second period before exposure to the ligand and the mean amplitude of current at −120 mV measured 1.5 to 2 minutes after the onset of exposure to the ligand. The chord conductance for the K$_\text{Na}$ current, which is shown in Figure 3C, was calculated by measuring the amplitude of the barium-sensitive current at −120 mV. The membrane potential of a sampled
cell monitored in the voltage-clamp mode was defined as the voltage at which the current was zero.

### Scanning Electron Microscopy

Standard techniques, as described previously, used to prepare glutaraldehyde-fixed isolated Müller cells for scanning electron microscopy, which was performed at the University of Michigan’s Anatomy and Cell Biology Core Facility.

### Chemicals

Chemicals were from Sigma (St. Louis, MO), unless otherwise noted.

### Statistics

Data are given as the mean ± SEM. Probability was evaluated by the Student’s t-test.

### RESULTS

Because the glutamate transporter of Müller cells is electrogenic, its function can be assessed by electrophysiological methods. In this study, the perforated-patch configuration of the patch-clamp technique was used to monitor the currents of isolated rat Müller cells. An advantage of the perforated-patch configuration is that it minimizes the disruption of the recorded cell’s cytoplasm and, consequently, the loss of intracellular regulatory molecules that may influence the functioning of the glutamate transporter. However, because a recording pipette detects currents generated by the activity of ion channels, as well as electrogenic transporters, we strove to minimize changes in ion channel activity during activation of transporter activity. To help in this, barium was included in the perfusate to block K$_{IR}$ channels, which are inhibited when Müller cells are exposed to glutamate. However, in Müller cells, glutamate also inhibits a barium-resistant outward current that is activated at voltages depolarized to approximately −50 mV. For this reason in a number of experiments, we used PDC, which is a selective ligand for the glutamate transporter. Because PDC potently binds to glutamate transporters as it is taken up into cells, this molecule is often used to prevent the transport of glutamate by GLAST. In our study, use of PDC permitted activation of the Müller cell glutamate transporter over a wide range of voltages without significant changes in ion channel activity.

### PDC-Induced Current in Rat Müller Cells

Initially, it was necessary to establish that the PDC-induced current in rat Müller cells has characteristics consistent with GLAST activity, because this ligand has not been used previously to monitor glutamate transporter function in mammalian Müller cells. Figure 2A shows the effect of PDC on the inward current monitored in a Müller cell freshly isolated from a control rat. Consistent with activation of GLAST, there was an increase in an inward (negative) current during exposure of the Müller cell to PDC. The PDC-induced current reversed rapidly when the perfusate was switched to one without PDC. As illustrated in Figure 2B, the PDC-induced current increased in amplitude with membrane hyperpolarization. Thus, similar to the electrogenic glutamate transporter observed in Müller cells from other species, the PDC current induced in rat Müller cells was dependent on the voltage across the cell membrane. Similar observations were made in 18 Müller cells isolated from normal retinas.

Another feature of GLAST is that transport is dependent on external sodium. Consistent with sodium dependence of the PDC-induced current in rat Müller cells, perfusion with a sodium-free solution eliminated the PDC-induced current (Fig. 2C). Similar observations were made in four Müller cells isolated from rat retinas.

A characteristic of GLAST is that the amplitude of the generated current is dependent on the concentration of the transported ligand. In isolated Müller cells, we found that the half-maximally effective concentration of PDC was approximately 10 μM (Fig. 2D). This is very similar to the value determined by Sarantis et al. for the activation by PDC of currents in salamander Müller cells. In addition, the half-maximally effective concentration of PDC is similar to that of glutamate when this amino acid was tested on salamander and rabbit Müller cells. Taken together, the results of these experiments are consistent with PDC’s activating GLAST in Müller cells freshly isolated from the rat retina.

### GLAST Current in Müller Cells of Diabetic Rats

To test the hypothesis that diabetes causes dysfunction of the Müller cell glutamate transporter, the GLAST current was quantified in Müller cells isolated from control and diabetic rats. For this series of experiments, we used the maximally effective PDC concentration (100 μM; Fig. 2D). Four weeks after administration of streptozotocin, the amplitude of the PDC-induced current had decreased by 36% (Fig. 3A). This was a significant (P = 0.005) decrease in GLAST current. At 13 weeks after injection, which was the maximum duration of diabetes studied, the amplitude of the PDC-induced current was decreased by 67% (P = 0.001), compared with the control amplitude.

Although, as reviewed earlier, there are advantages to using PDC to selectively activate GLAST over a wide range of voltages, it was of interest to confirm an effect of diabetes on the transport of glutamate, which is the in vivo ligand for this transporter. By measuring the glutamate-induced current at a hyperpolarized voltage (~120 mV) in the presence of barium, which inhibits the glutamate-sensitive K$_{IR}$ channels, GLAST currents were selectively activated by its physiological ligand. As illustrated in Figure 3B, the glutamate-induced inward current was significantly (P = 0.004) diminished in Müller cells from retinas of diabetic rats. This finding with glutamate is consistent with the observed decrease in the PDC-induced currents in Müller cells from diabetic retinas. Thus, it appears that early in the course of diabetes the glia have a diminished capability for removing glutamate from the extracellular space.

We considered the possibility that the activity of glutamate transporter was decreased in diabetic Müller cells due to a generalized disturbance in physiology of these glia (Fig. 3C). However, this seems unlikely, because the membrane potentials of the sampled cells were not significantly (P > 0.3) different in the control and diabetic groups. In addition, the amplitude of the K$_{IR}$ current, which is the predominant ionic current of Müller cells, was not significantly (P > 0.16) different in the Müller cells isolated from diabetic and control rats. An alternative possibility to account for the reduction in the amplitude of the current generated by the glutamate transporter is that diabetes causes Müller cells to become smaller. With a smaller surface area, there may be fewer GLAST molecules per cell and, thereby, a smaller current generated as glutamate is transported across the membrane. However, measurements of cell membrane capacitance, which is an indicator of cell size, did not reveal a significant (P > 0.05) change from control levels during 13 weeks of streptozotocin-induced diabetes. Thus, the observed decrease in the activity of the glutamate transporter was most likely not part of a generalized physiological deterioration of Müller cells. Rather, it appears that early in diabetic retinopathy there is a selective vulnerability of this transporter molecule.
Oxidation and GLAST Function in Diabetic Müller Cells

We also sought to identify the mechanism by which diabetes causes dysfunction of the glutamate transporter in Müller cells. Experiments were performed to help test the hypothesis that oxidative mechanisms play a role. This seems to be a reasonable idea, because diabetes is associated with oxidative stress47 and the cloned GLAST molecule possesses redox-regulatory elements.32

To help to test the hypothesis that GLAST dysfunction in diabetic Müller cells is caused by oxidative processes, the effect of a chemical reductant was tested. If a chemical reducing agent reversed the dysfunction of this transporter, then this would be support for the idea that the Müller cell glutamate transporter in the diabetic retina was in an oxidized state. Based on this reasoning, the effect of the disulfide-reducing agent, disulfide dithiothreitol (DTT), was assessed.

As illustrated in Figure 4A, exposure of a diabetic Müller cell to DTT promptly and markedly increased the amplitude of the inward current induced by PDC. Subsequent exposure of this Müller cell to PDC alone (without DTT) resulted in a response that was similar in amplitude to the current that had been induced during exposure to PDC plus DTT. The persistent restoration of transporter activity is consistent with DTT's having chemically modified the glutamate transporter—that is, changed this transporter molecule from being in an oxidized state to being in a reduced state.

In a series of experiments, the effect of DTT was tested on control Müller cells and those isolated from rats that had been diabetic for various lengths of time (Fig. 4B). Exposure to this disulfide-reducing agent completely reversed the diabetes-associated decrease in the amplitude of the PDC-induced current. This effect was not due to DTT itself inducing a current that was independent of PDC, because the basal currents of Müller cells did not change significantly (P > 0.05, n = 5) when the perfusate contained DTT without PDC. Thus, the DTT-mediated recovery of glutamate transporter activity in diabetic Müller cells is consistent with this transporter molecule’s being oxidized in the diabetic retina.

Because studies of cloned GLAST have demonstrated that oxidation diminishes transporter function without altering the half-maximally effective concentration of glutamate,31 the dose–response relationship for the current induced in Müller cells during exposure to PDC was assessed. As shown in Figure 4C, the half-maximally effective concentration of glutamate was approximately 8 μM in both the control and experimental groups. Of further interest, this finding is almost identical with the half-maximally effective concentration re-
Glutamate Transport in Diabetic Müller Cells

FIGURE 3. Effect of diabetes on the amplitude of the GLAST current in Müller cells. (A) Currents induced by PDC were measured in Müller cells isolated from control and diabetic rats. Note that the more negative the current, the greater the PDC-induced transporter activity. Over the 13-week course of streptozotocin-induced diabetes, the blood glucose levels before death were not significantly (P > 0.1) different: The mean level of blood glucose was 378 ± 6 mg/dL in the diabetic rats. A mean of 9 ± 2 Müller cells was sampled for each time point. (B) Effect of diabetes on the glutamate-induced current in Müller cells. Mean induced currents in a series of 11 Müller cells from control rat retinas and 8 cells isolated from rats having diabetes for 9 ± 1 weeks. Note that the greater the glutamate-induced activation of the transporter, the more negative the current. (C) Physiological parameters of retinal Müller cells obtained from rats at various times after streptozotocin injection. Left: membrane potential; middle: conductance of the K+ current; and right: cell membrane capacitance. For each panel, a mean of 8 ± 1 Müller cells was sampled for each data point. (A, B) Significant (P < 0.005) decrease compared with control levels. Soon after the onset of experimental diabetes, the amplitude of the GLAST inward current was selectively decreased.

ported for the glutamate transporters of Müller cells from salamanders42 and rabbits,43 the only other species studied previously. Thus, similar to the reported effect of oxidation on cloned GLAST molecules, diabetes was associated with a significant decrease in the maximal activity of the Müller cell glutamate transporter, but did not alter the half-maximally effective concentration of glutamate, at least during the initial 13 weeks of experimental diabetes. Taken together, our experimental results support the hypothesis that early in the course of diabetic retinopathy the function of the glutamate transporter in retinal Müller cells is decreased by a mechanism involving oxidation.

**DISCUSSION**

The results of this study show that the maximal activity of the glutamate transporter in rat Müller cells decreases by two thirds during the initial 13 weeks of streptozotocin-induced diabetes. Consistent with this dysfunction’s being caused by an oxidative process, exposure of diabetic Müller cells to a disulfide-reducing agent rapidly and fully restored the activity of this transporter. Thus, the experimental findings of this study support the hypothesis that soon after the onset of diabetes the function of the glutamate transporter in retinal Müller cells is decreased by a mechanism involving oxidation.

The finding that the activity of the Müller cell glutamate transporter is decreased in experimental diabetes provides new insight into putative mechanisms that may account for observations made in previous studies of the diabetic retina. For example, a decrease in the ability of Müller cells to transport glutamate from the extracellular space is likely to cause levels of this amino acid to increase in the microenvironment. In agreement with this prediction, Lieth et al.28 and Kowluru et al.29 reported that the concentration of retinal glutamate is elevated early in the course of experimental diabetes. Also, the finding by Ambati et al.30 of raised glutamate levels in the vitreous of patients with diabetic retinopathy suggests that diabetes is associated with an elevated concentration of this amino acid in the human retina.

Recent observations suggest that the mechanism by which glutamate levels are elevated in the diabetic retina may involve oxidative stress. For example, Kowluru et al.28 documented that diabetic rat retinas with increased glutamate levels also had a 100% increase in thiobarbituric acid-reactive substances (TBARS), which are indicators of oxidative stress. Further support for a link between oxidative stress and elevated glutamate levels in the diabetic retina is that treatment of diabetic rats with a mixture of antioxidants blocked both the increase in TBARS and the increase in the concentration of glutamate in the retina. Taken together, the findings of Kowluru et al.28 suggest that oxidative stress causes an increase in glutamate levels in the diabetic retina.

How are oxidative stress and glutamate homeostasis linked? The findings presented in this study indicate that one mechanism by which oxidative stress may increase the levels of glutamate in the diabetic retina involves an inhibition of the Müller cell glutamate transporter. Inhibition of this transporter compromises the ability of Müller cells to remove glutamate from the extracellular space. As a result, the levels of this amino acid in the retinal microenvironment would increase. Thus, based on recent reports and the experimental findings of this study, a likely scenario is that an oxidation-induced inhibition of GLAST molecules in Müller cells contributes significantly to the disruption of glutamate homeostasis in the diabetic retina.

Dysfunction of the glutamate transporter in Müller cells may augment the level of oxidative stress in the diabetic retina. Support for this possibility is that glutamate enhances the generation in the retina of oxidative stress, as indicated by an increase in TBARS.29 Pharmacologic experiments indicate that the glutamate-induced increase in retinal oxidants involves the activation of N-methyl-D-aspartate receptors and the production of nitric oxide (NO).28 In an environment of excess reactive oxygen species and oxidative stress, NO is readily converted into potent oxidants that can cause dysfunction of GLAST molecules.47 These observations suggest that in the retina there is a positive feedback loop involving glutamate and oxidative stress. By failing to maintain glutamate homeostasis in the diabetic retina, Müller cells may play a key role in augmenting oxidative stress.

A positive feedback loop involving oxidative stress and dysfunction of GLAST molecules in Müller cells may be important in the progression of diabetic retinopathy (Fig. 5). This idea is based on the emerging concept48 that oxidative stress initiates the four main molecular mechanisms implicated in the pathogenesis of diabetic complications (i.e., increased polyol synthesis, formation of advanced glycation end-products, activation of protein kinase C, and enhanced flux through the hexosamine pathway). Thus, inhibition of the glutamate trans-
porter in Müller cells may augment oxidative stress in the retina and may thereby be an important step in the development of sight-threatening complications in eyes with diabetic retinopathy.

As with any study, there are limitations in the interpretation of the experimental results. For example, although streptozotocin-injected rats provide an intensively studied model of diabetes, it is not identical with the clinical situation. One reason for this is that uncontrolled hyperglycemia is not typical in patients with type 1 diabetes. Also, rats may respond differently to hyperglycemia and hypoinsulinemia than humans. Additionally, a chemically induced loss of B cell function is very rare in humans. However, the possibility of a direct toxic effect of streptozotocin on Müller cells seems unlikely in this study, because the membrane potential, predominant ionic conductance, and cells size were not significantly changed in the sampled cells during 13 weeks of experimental diabetes. In addition, the rapid and complete recovery of glutamate transporter activity during exposure to a chemical reducing agent makes an oxidative effect a more likely explanation for the transporter dysfunction than nonspecific damage caused by streptozotocin. Thus, despite some limitations, the observations made in this study support our hypothesis that oxidation compromises the function of the glutamate transporter in diabetic Müller cells.

An additional experimental limitation is the use of Müller cells isolated from the retina. Because an in vivo application of the electrophysiological technique used in this study seems impractical at present, it remains to be demonstrated that oxidation compromises the function of the glutamate transporter in diabetic Müller cells.

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FIGURE 4. Evidence that oxidation accounts for the decreased GLAST activity in diabetic Müller cells. (A) Effect of the reducing agent DTT on the current induced by PDC in a Müller cell from a diabetic retina. Current amplitudes were measured at 10-second intervals in a Müller cell isolated from a rat made diabetic by streptozotocin injection 6 weeks earlier. Bars: times during which the perfusate contained 100 μM PDC (solid lines) and 3 mM DTT (dashed line). (B) Summary of the effect of DTT during 13 weeks of streptozotocin-induced diabetes. For each value, a mean of 9 ± 2 cells was sampled. *Significant (P < 0.001) increase in the PDC-induced current during exposure to DTT. (C) Dose–response relationship for the glutamate-induced inward current in control Müller cells (○, n = 4) and in Müller cells from rats that were diabetic for 9 weeks (●, n = 4). Because glutamate transporter activity in diabetic Müller cells was decreased relative to control levels (Fig. 3B), the amplitudes of currents in the two groups were normalized to facilitate comparison of half-maximally effective concentrations. Early in the course of diabetic retinopathy, the function of the glutamate transporter in Müller cells was decreased by a mechanism that is likely to involve oxidation.
physiological methods are perfected to assay Müller cells in the diabetic retina in vivo, information gained from the study of isolated Müller cells will help in the design of experiments to assess the functional effects of diabetes on these glia.

Dysfunction of the Müller cell glutamate transporter is one of the earliest reported diabetes-induced changes in these glia. For example, this change precedes the decrease in activity of glutamine synthetase, which is the rate-limiting enzyme for the conversion of glutamate to glutamine in Müller cells. This enzyme’s activity is not affected until after 8 weeks of experimental diabetes.49 Similarly, it is not until 6 to 8 weeks of experimental diabetes that there is an upregulation in the expression of glial fibrillary acidic protein, 9 which is an intermediate filament of uncertain function that is expressed by Müller cells in response to a multitude of retinal perturbations. Thus, the glutamate transporter of Müller cells appears to be a molecule that is particularly vulnerable early in the course of diabetes.

The demonstration in this study that the diabetes-induced dysfunction of the Müller cell glutamate transporter can be rapidly reversed, at least early in the course of diabetic retinopathy, renders GLAST a potential target for pharmacologic intervention. By enhancing the ability of Müller cells to regulate glutamate levels in the diabetic retina, it may be possible to prevent or diminish subsequent molecular events that lead to sight-threatening complications.

References


