Antisense Knockdown of GLAST, a Glial Glutamate Transporter, Compromises Retinal Function

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Purpose. To elucidate the role of the glial glutamate transporter GLAST, in the regulation of retinal function.

Methods. Antisense oligonucleotides to GLAST were injected intravitreally into the left eye of Wistar rats. Sense oligonucleotides (control) were injected into the right eye over a period of 3 days. Scotopic flash electroretinograms were recorded over a 20-day period. To assay whether the antisense oligonucleotides caused a reduction in the expression or the activity of GLAST, retinas were exposed to D-aspartate, a nonendogenous substrate of glutamate transporters. The retinas were immunolabeled with specific antibodies for D-aspartate. Retinal GLAST and glutamate distributions also were determined immunocytochemically.

Results. Antisense oligonucleotides markedly suppressed the electroretinogram b-wave, whereas sense oligonucleotides had no significant effect. Significant changes in the electroretinogram were apparent 5 days after injection of antisense oligonucleotide and were sustained for at least 20 days. A marked reduction of D-aspartate uptake into Müller cells of retinas that had been exposed to the antisense oligonucleotides 5 days previously suggests a reduction of GLAST activity. The retinas, however, displayed no evidence of excitotoxic neuronal degeneration, and the distribution of glutamate was unaffected by antisense treatment.

Conclusions. The observed lack of neuronal degeneration suggests that reduced glutamate uptake into Müller cells does not cause excitotoxic tissue damage. A direct perturbation of glutamatergic signaling is more likely, because the rapid clearance of glutamate is necessary for light elicited signaling between photoreceptors and bipolar cells. This suggests that GLAST is essential for the maintenance of normal retinal transmission. (*Invest Ophthalmol Vis Sci.* 2000;41:585–591)

Glutamate is the major excitatory neurotransmitter in the retina.[1,2] The homeostasis of extracellular levels of glutamate is critical for retinal function under normal and pathologic conditions. This task is performed by a number of excitatory amino acid transporters (EAATs) localized to neuronal and glial elements. Five distinct human EAATs (EAAT1–5) have been cloned.[3–5] Rodent homologues of EAATs 1 to 4 have been cloned, and EAAT1 (GLAST), EAAT2 (GLT-1), and EAAT 3 (EAAC-1) have been identified by immunocytochemistry in the rodent retina.[6,7] GLT-1 is predominantly localized to astrocytes in the brain, but immunocytochemical analysis of the rat retina has shown GLT-1 to be associated with cones and cone bipolar cells, not glial elements.[8] EAAC-1 is associated with rat retinal neurons whose processes ramify in the inner plexiform layer, principally amacrine cells.[9] The cellular localization of EAAT5 in the retina has not been established unequivocally. EAAT5 has been reported to be associated with Müller cells in the salamander retina[10] and photoreceptors in the rat retina.[11] GLAST is exclusively associated with Müller cells rather than neurons,[6,12] but GLAST-immunopositive Müller cell processes do ensheath the somata of most photoreceptors, bipolar cells, and ganglion cells.[6] Autoradiographic studies indicate that Müller cells dominate total retinal glutamate transport, utilizing GLAST.[13–15] GLAST is involved in the neuron–glia glutamate–glutamine cycle whereby Müller cells take up neuronally released glutamate and convert the glutamate to glutamine. Neuronal function is thought to be dependent on the availability of glutamine released by the Müller cells.[16]

Because specific inhibitors of glutamate transport do not exist, we investigated the role of GLAST by the inhibition of its synthesis with antisense oligonucleotides. Similar techniques have been used to knockout GLAST in the striatum and hippocampus.[17] Furthermore, antisense administration has been shown to be a highly effective approach for the regulation of protein expression in the retina.[18,19]

Materials and Methods

Animals

Female Wistar rats (200–250 g) were used for all experiments. All animals were treated in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and were within the ethical guidelines of the Australian National Health and Medical Research Council and The University of Queensland.

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Oligonucleotides

Antisense and sense oligonucleotides corresponding to nucleotides −12 to 15 of the cloned rat glutamate transporter, GLAST[5] were commercially synthesized (Pacific Oligos, Brisbane, Australia). The antisense sequence GTTGCTTTTTGTCATATTTTATCTTTC and the sense sequence GAAAGATAAAATATGACAAAAAGCAAC were protected by the use of phosphorothioate linkages between each of the bases. This modification also increases lipid solubility. The left eye of each rat was injected on day 1 and day 3 of the experiment with 2 μl of sterile water containing 30 μg of antisense oligonucleotide. The right eye served as a control and was injected with sense oligonucleotide.

Electroretinography

Rats were anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (12 mg/kg), placed in a stereotaxic frame, and maintained at 37°C with a homeothermic blanket. The rat's pupils were dilated with 1% tropicamide, and the corneas kept moist with carmellose sodium (Celluvisc; Allergan, Sydney, Australia). The animals were then allowed to dark-adapt for 30 minutes before the recording of scotopic flash electroretinograms (ERG). A platinum wire loop was placed on each cornea to act as the recording electrode, and a reference electrode was connected to each ear. Ground electrodes were attached to the animal's back. A xenon strobe light placed 0.5 m in front of the animal presented the flash stimulus at 0.25 Hz. Eight consecutive responses were amplified and averaged using a MacLab/2e bioamplifier/data recorder running "Scope" software (AD Instruments, Sydney, NSW, Australia). The peak amplitudes of the a- and b-waves of the electroretinogram were determined before the injection of sense or antisense oligonucleotides and at 5 (n = 13), 10 (n = 7), and 20 (n = 5) days after injection. The a-wave amplitude was measured from the baseline to the trough of the a-wave response, and the b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave. Data are expressed as the mean wave amplitude ± SEM (μV). Two-factor repeated-measures analysis of variance (ANOVA) was performed to compare the responses from antisense-treated retinas with those from sense-treated control retinas over the 20-day period. Implicit times were measured from the flash stimulus to the peak of the b-wave response.

Glutamine Effects on the Suppressed ERG

After the suppression of the ERG by antisense oligonucleotides, the effect of an intraocular injection of glutamine was investigated. Glutamine (4 mM in 2 μl saline) was injected 5 days after the first GLAST antisense injection. Electroretinograms were recorded 5 and 20 minutes after glutamine administration.

Immunocytochemistry for GLAST

Five days after the initial injection of antisense or sense oligonucleotides, rats were killed by an overdose of sodium pentobarbital [200 mg/kg intraperitoneally (IP)]. Isolated retinas were immersion fixed for 90 minutes with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The fixed retinas were embedded in 4% agar and 30-μm-thick transverse sections cut on a Vibratome (T.P.I., St. Louis, MO). Free-floating sections were labeled with antisera raised in rabbit against the N terminus of GLAST[6] and diluted 1:100,000 in phosphate-buffered saline containing 0.2% Triton X-100 plus 5% normal horse serum. Labeling was detected using donkey anti-rabbit (Amersham, Sydney, NSW, Australia) diluted 1:300, followed by streptavidin–biotin–horseradish peroxidase complex (Amersham) diluted 1:300. Diaminobenzidine was used as a chromogen.

D-Aspartate Uptake and Immunolabeling

Rats were killed by an injection of sodium pentobarbital (200 mg/kg IP), and the eyes were enucleated immediately. Isolated retinas were incubated in vitro at 37°C for 90 minutes in oxygenated Ames medium containing D-aspartate (50 μM), a nonendogenous substrate of glutamate transporters that can be detected by immunocytochemistry.[20] The retinas were then fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 16 hours, dehydrated, and embedded in Araldite (ProSciTech, Thuringowa, QLD, Australia) resin. Semi-thin sections (0.5 μm thick) of retina were then immunolabeled with specific antibodies for D-aspartate (1:30,000 dilution)[6] or glutamate (1:300,000) using our standard techniques with previously characterized antisera.[21]

Morphometric Analysis

Semi-thin sections of retina obtained approximately 2 mm superior to the optic disc were stained with toluidine blue, viewed on a Zeiss Axioskop microscope (Oberkochen, Germany), and digitally imaged. The thickness of the inner plexiform layer, the inner nuclear layer, the outer nuclear layer, and the total retinal thickness were analyzed using NIH Image software. The retinas of six rats were measured, and a paired t-test was used to compare values obtained from antisense-treated eyes with those from control, sense-treated eyes.

Results

GLAST Immunocytochemistry

Five days after the initial injection of GLAST sense oligonucleotide, the distribution and intensity of GLAST immunoreactivity was indistinguishable from that seen in normal adult rat retinas (Fig. 1A, 1B). Intense staining was seen in the glial elements of the retina, particularly the Müller cell end feet forming the outer limiting membrane. Three distinct bands of GLAST immunolabeling were seen in the inner plexiform layer, and heavy labeling of the outer plexiform layer, corresponding to the lateral processes of the Müller cells. Moreover, Müller cell processes ramifying through the inner nuclear layer ensheath the bipolar cell perykarya. Positively labeled radial Müller cell processes could be seen throughout the outer nuclear layer. No GLAST-like immunoreactivity was evident in neuronal elements of the retina.

The intensity of GLAST immunoreactivity was drastically reduced by the injection of GLAST antisense oligonucleotides, indicating that knockdown of GLAST was successful (Fig. 1C). After 5 days, the labeling in the plexiform layers and that of the Müller cell end feet was very weak, but still observable. Weakly labeled radial Müller cell processes also could be seen in the outer nuclear layer. It was no longer possible, however, to distinguish the Müller cell processes ensheathing the bipolar cell bodies in the inner nuclear layer, where all GLAST expression appeared to be

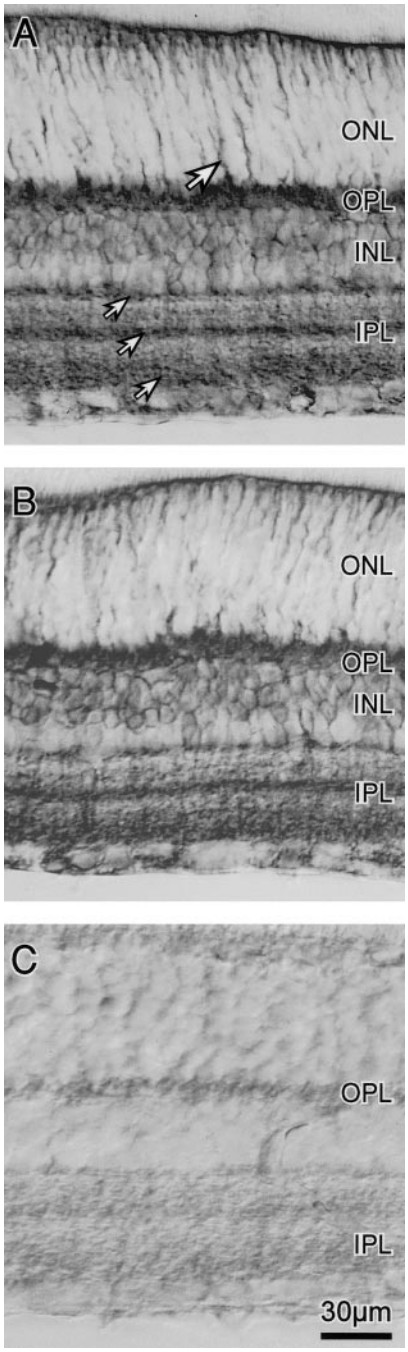

FIGURE 1. Rat retinas immunolabeled for GLAST. **(A)** In a normal control retina, labeling is associated with Müller cells. Radial processes (*large arrow)* and end feet in the outer nuclear layer (ONL) are labeled, as is a dark band in the outer plexiform layer (OPL). Cells in the inner nuclear layer (INL) are surrounded by GLAST-immunoreactive processes. Three bands (*small arrows)* of lateral processes within the inner plexiform layer (IPL) are immunolabeled for GLAST. **(B)** The pattern of GLAST-immunoreactivity is not affected by the administration of GLAST sense oligonucleotides. **(C)** Five days after GLAST antisense treatment, the expression of GLAST is drastically reduced. Weak GLAST immunoreactivity can be seen in the OPL and IPL.

suppressed. Furthermore, antisense oligonucleotide treatment significantly reduced GLAST labeling of astrocytes/Müller cell end feet in the ganglion cell layer/nerve fiber layer, when compared with sense treated control retinas.

GLAST Activity

The activity of the GLAST transporter was assessed before and after oligonucleotide treatment by measuring the accumulation of D-aspartate by immunocytochemistry, since D-aspartate is a substrate for all glutamate transporters. No endogenous D-aspartate immunolabeling is observed in retinas that are fixed immediately and had not been exposed to exogenous D-aspartate.[6] Following incubation with D-aspartate of normal retinas that had not been treated with oligonucleotides, D-aspartate was exclusively accumulated into the Müller cells (Fig. 2A). The strongest labeling occurred in the Müller cell perykarya, the end feet in the nerve fiber layer, and the outer limiting membrane. No D-aspartate immunoreactivity was observed in any neuronal cells. A similar pattern of D-aspartate accumulation by the Müller cells was seen in retinas that had been injected with sense oligonucleotides 5 days previously (Fig. 2B).

Five days after the first intraocular antisense injection, the pattern of D-aspartate accumulation and immunoreactivity altered significantly. The transporter substrate accumulated in the photoreceptors, bipolar cells, and a few ganglion cells, whereas no significant D-aspartate immunolabeling could be observed in the glial Müller cells. The bipolar cell terminals in the ganglion cell layer displayed particularly intense D-aspartate staining (Fig. 2C). Ten days after the initial injection of GLAST antisense, the Müller cells regained their ability to accumulate D-aspartate. Although the D-aspartate localized to the Müller cells, the intensity of labeling was much weaker than that of the preinjected or control sense-treated retinas. The D-aspartate appeared to be transported to the glial cell bodies, which stained more intensely than their radial processes and end feet. Very little D-aspartate immunoreactivity could be seen in neuronal elements 10 days after antisense oligonucleotide treatment (Fig. 2D).

Glutamate Immunocytochemistry

In sense-injected control retinas, glutamate immunoreactivity was observed in the inner segments of photoreceptors, as well as in bipolar cells and their processes in the inner plexiform layer (Fig. 3A). The somata of ganglion cells also were strongly labeled for glutamate. No labeling was observed in the processes or somata of Müller cells. The distribution and intensity of glutamate labeling was unaffected by the administration of GLAST antisense, when observed 5 or 10 days after the first injection of oligonucleotide (Fig. 3B).

Morphometric Analysis

Table 1 shows that retinal thickness was not affected by GLAST antisense treatment. No significant neurodegeneration or edema was observed by morphometric analysis of the inner plexiform layer, inner nuclear layer, and outer nuclear layer performed 10 days after the initial injection of oligonucleotide.

Histologic observation of toluidine blue–stained 0.5-μm sections of retina revealed little difference between the antisense- and sense-treated control retinas. All neurons appeared to be normal, with no evidence of excitotoxic neuronal damage. Swelling of the Müller cells was, however, evident 10 days after the antisense treatment and can be seen clearly in Figures 3B and 4B.

Electroretinography

The intraocular injection of GLAST sense oligonucleotide did not affect the waveform of the scotopic flash ERG. It can be

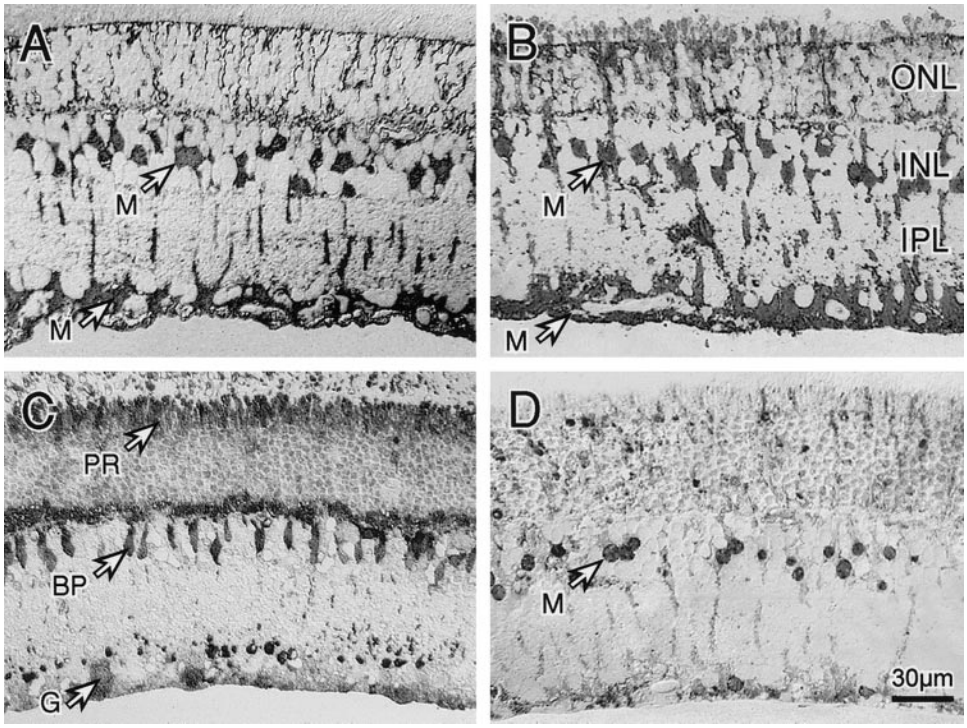

FIGURE 2. Rat retina exposed to D-aspartate (50 μM, in vitro) and then immunolabeled for the presence of D-aspartate. **(A)** D-Aspartate is taken up exclusively by Müller cells (M) in control retinas. **(B)** The pattern of D-aspartate accumulation is not affected by the administration of GLAST sense oligonucleotides. **(C)** Five days after the inhibition of GLAST synthesis by antisense oligonucleotides, there is a marked reduction in D-aspartate uptake by Müller cells and the appearance of D-aspartate in photoreceptors (PR), bipolar cells (BP), and ganglion cells (G). **(D)** Ten days after antisense administration, weak D-aspartate uptake and immunolabeling is associated with Müller cells (M). Outer nuclear layer, ONL; inner nuclear layer; INL; inner plexiform layer, IPL.

seen in Figure 5 that neither the amplitude nor the latency of the positive-going b-wave were altered. Single-factor ANOVA showed no significant change in the b-wave amplitude over 20 days after the injection ($F_{(3)} = 1.10$, $P = 0.36$). Similarly, the negative-going a-wave was not affected by the administration of sense oligonucleotides ($F_{(3)} = 0.82$, $P = 0.49$). The sense-injected eye was subsequently used as the control for the contralateral antisense-injected eye in all experiments.

Figure 6 shows that the b-wave amplitudes of eyes that had been injected with antisense oligonucleotides to GLAST were significantly reduced when compared with ERGs recorded from sense-treated control eyes (two-factor repeated-measures ANOVA, $F_{(78)} = 7.80$, $P = 0.007$). Post hoc, paired *t*-tests revealed a significant ($P < 0.01$) suppression of the b-wave at 5, 10, and 20 days after injection. Five days after the first antisense injection, the mean b-wave amplitude was 169 ± 31 μV ($n = 13$) compared with 342 ± 64 μV (sense control); at 10 days the mean b-wave was 192 ± 49 μV (274 ± 71 μV sense control, $n = 7$), and after 20 days, the amplitude was reduced to 107 ± 32 μV (283 ± 88 μV sense control, $n = 5$).

The implicit time of the b-wave component was not affected by the intraocular injection of GLAST antisense oligonucleotides ($F_{(78)} = 0.00004$, $P = 0.995$). Furthermore, the apparent decrease in the a-wave amplitude after administration of GLAST antisense oligonucleotides seen in Figure 7 is not statistically significant when compared with sense-treated control values ($F_{(78)} = 0.85$, $P = 0.36$).

The intraocular injection of glutamine (4 mM) after antisense treatment had no apparent effect on the amplitude nor on the waveform of the electroretinogram when recorded 5 minutes or 20 minutes after administration (Fig. 8).

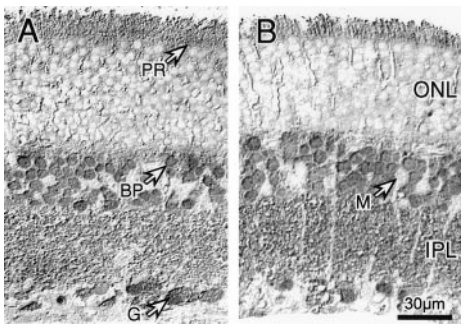

FIGURE 3. Glutamate immunoreactivity in the rat retina. **(A)** In sense-injected control retina, glutamate is associated with the inner segments of photoreceptors (PR), bipolar cells (BP), and ganglion cells (G). **(B)** The pattern of glutamate labeling is not altered when observed 5 or 10 days after the injection of GLAST antisense oligonucleotides. The inhibition of GLAST synthesis does, however, cause swelling of the Müller cells (M). Outer nuclear layer, ONL; inner plexiform layer, IPL.

DISCUSSION

We have recently shown that changes in GLAST expression are temporally associated with periods of cellular differentiation

TABLE 1. Morphometric Analysis of Retinal Layers 10 Days after Oligonucleotide Administration

	Thickness (μm)			
Layer	**Sense**	**Antisense**	***n***	***P***
IPL	38 ± 3	40 ± 2	6	0.61 (NS)
INL	29 ± 2	32 ± 3	6	0.42 (NS)
ONL	40 ± 5	45 ± 3	6	0.37 (NS)
Total	138 ± 12	159 ± 11	6	0.23 (NS)

Values are means ± SEM. NS, not statistically significant ($P > 0.05$).

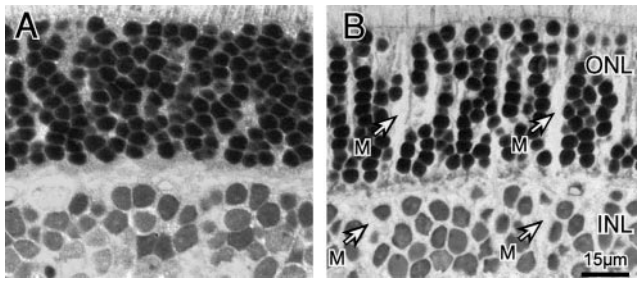

FIGURE 4. Semi-thin sections of rat retina stained with toluidine blue. **(A)** Ten days after GLAST sense oligonucleotide administration, the histology of the outer retina appears to be normal. **(B)** Ten days after the injection of GLAST antisense oligonucleotide, edematous Müller cells (M) are apparent in the inner nuclear layer (INL), and their swollen processes are evident in the outer nuclear layer (ONL).

and synaptogenesis.[6] This study demonstrates that electrical function of the adult rat retina is also dependent on functional GLAST transporters. Because of the unavailability of specific glutamate transporter inhibitors, antisense oligonucleotides of GLAST were used to suppress the synthesis of new retinal GLAST protein in vivo. Figure 1 shows that this approach successfully reduces the expression of GLAST in the retina, as evinced by immunocytochemistry. Moreover, the small degree of variation between the ERGs recorded from different animals (Fig. 6) suggests that this intraocular antisense approach is particularly applicable to the targeting of glial cell proteins, as astrocytes and Müller cell end feet form the vitreous/retinal barrier. The suppression of retinal GLAST expression has previously been shown in a knockout mouse model,[22] but the method of direct intraocular injection of oligonucleotides used in this study targets the effect to the retina, eliminating the possibility of global effects.

We demonstrate directly that the inhibition of GLAST synthesis alters the capacity of Müller cells to transport D-

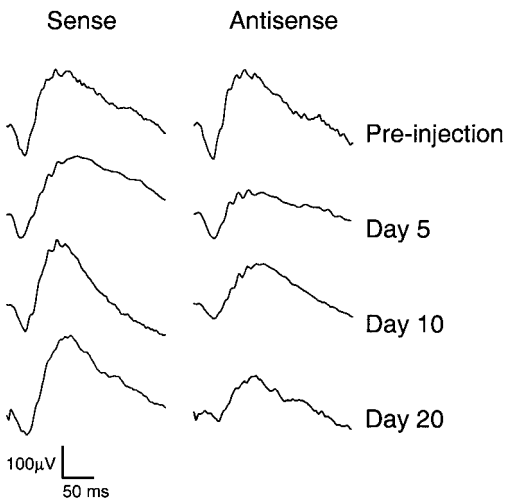

FIGURE 5. Scotopic electroretinograms (ERG) recorded before (Pre-injection) and 5, 10, and 20 days after the intraocular injection of either GLAST sense or antisense oligonucleotides. Administration of GLAST sense has no effect on the ERG. Five days after antisense administration, the ERG b-wave is reduced. The b-wave suppression remains for at least 20 days.

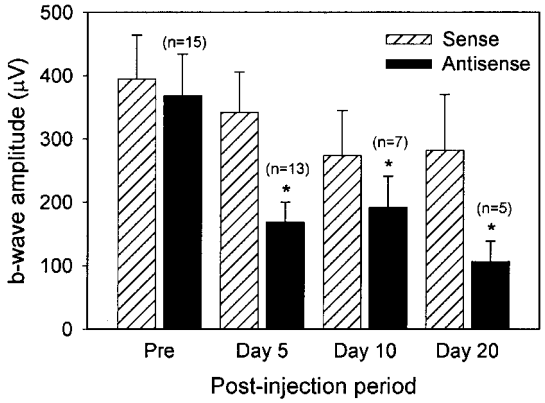

FIGURE 6. The effect of GLAST sense and antisense oligonucleotides on the ERG b-wave amplitude. The administration of GLAST antisense significantly reduces the ERG b-wave amplitude when compared with the GLAST sense-treated contralateral control eye response. Each bar represents the mean ± SEM. *Statistically significant ($P < 0.01$) difference between the mean antisense and sense responses.

aspartate, and by inference, glutamate. Five days after the antisense oligonucleotide injection, the Müller cells lose all ability to accumulate exogenous D-aspartate (Fig. 2C). Müller cell processes ensheath retinal neurons and provide a physical barrier that normally prevents the uptake of D-aspartate into neurons, even though the neurons possess the glutamate transporters, GLT-1 and EAAC-1.[8,9] Inhibition of Müller cell GLAST synthesis removes the barrier to neuronal D-aspartate uptake and results in the pattern of D-aspartate labeling seen in Figure 2C. D-aspartate is now accumulated into photoreceptors, bipolar cells, and ganglion cells. Moreover, the lack of detectable D-aspartate uptake by Müller cells supports the view that GLAST is the overwhelmingly predominant glutamate transporter in Müller cells.[6] It is unlikely that any other glutamate transporters, such as EAAT5, if expressed in Müller cells,[10] make any significant functional contribution to glutamate homeostasis by the Müller cells. The accumulation of D-aspartate by neurons rather than Müller cells after the antisense administration is, however, transient. Ten days after the first oligo-

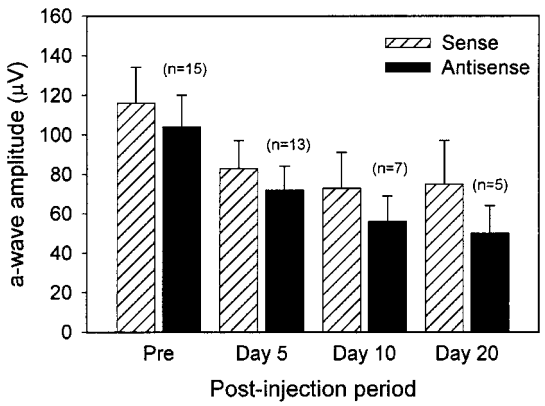

FIGURE 7. The effect of GLAST sense and antisense oligonucleotides on the ERG a-wave amplitude. GLAST antisense oligonucleotides do not have a statistically significant effect on the a-wave amplitude when compared with responses obtained from sense-treated control retinas. Each bar represents the mean ± SEM.

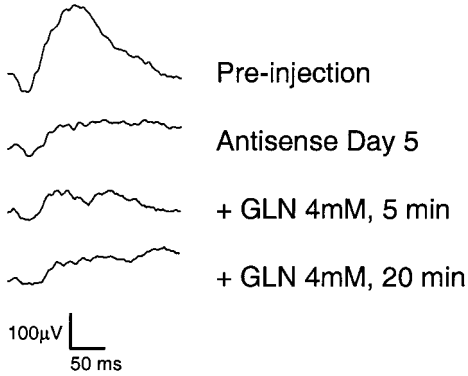

FIGURE 8. Scotopic electroretinograms recorded before (Pre-injection) and 5 days after the administration of GLAST antisense oligonucleotides. When the ERG b-wave was maximally suppressed at day 5, glutamine (GLN, 4 mM) was injected intraocularly. Glutamine had no effect on the ERG when recorded 5 or 20 minutes after injection.

nucleotide injection (and 7 days after the second, final injection), exogenous D-aspartate is accumulated weakly into Müller cells (Fig. 2D). This observation is indicative of a return of GLAST synthesis.

The onset of GLAST synthesis by Müller cells does not, however, result in a return of normal retinal function. The ERG b-wave, which is significantly attenuated 5 days after antisense administration, remains suppressed for at least 20 days (see Fig. 6). The suppression of the ERG b-wave, but not of the a-wave, observed in this study is in agreement with the electrophysiological findings reported by Harada et al.,[22] who used a GLAST knockout mutant mouse model. A number of possibilities could explain the inhibition of the ERG b-wave resulting from the perturbation of glutamate homeostasis. Glutamatergic neurons such as photoreceptors and bipolar cells are dependent on a supply of glutamine from Müller cells to synthesize glutamate for neurotransmission.[16] This glutamine is synthesized by the glial enzyme, glutamine synthetase, from accumulated glutamate. It is a possibility that after the inhibition of GLAST expression, the supply of glutamine to the neurons is cut off because of the unavailability of its precursor glutamate in the Müller cells. It is unlikely, however, that inadequate glutamine availability is the cause of the ERG b-wave suppression reported here. Intraocular glutamine administration had no effect on the ERG when injected on day 5, that is, when the ERG was maximally suppressed (Fig. 8). Moreover, the pattern of glutamate immunoreactivity is not altered by GLAST antisense treatment (Fig. 3). Thus, the suppression of the ERG b-wave is not likely to be due to an exhaustion of neuronal glutamate. The specificity of the oligonucleotides ensures that the neuronally localized glutamate transporters are unaffected and thus remain capable of taking up sufficient glutamate to maintain intracellular neuronal glutamate levels (Figs. 2, 3). Furthermore, because D-aspartate is transported into photoreceptor and bipolar cells, but not into Müller cells, 5 days after antisense administration (Fig. 2), it is likely that neuronal reuptake rather than Müller cell glutamate glutamine cycling[16] is responsible for replenishing the transmitter pool of glutamate. A more plausible explanation for the observed physiological effects is an increase in the concentration of extracellular glutamate due to compromised transporter activity. The rapid removal of glutamate from the extracellular space is vital for neurotransmission from photoreceptors to bipolar cells. A rise in extracellular glutamate concentration at this synapse, which could occur in the absence of GLAST activity, would block ON-bipolar cell depolarization and hence inhibit the generation of the ERG b-wave.[23] However, this idea is complicated by the finding that the ERG b-wave remains suppressed for 20 days after the antisense treatment, even though D-aspartate, and by inference glutamate, uptake into Müller cells returns after 10 days. Although morphometric analysis revealed no significant changes in retinal thickness, histologic alterations of Müller cells could be seen clearly after antisense administration (Fig. 4). Although the sustained increase in extracellular glutamate concentration did not result in obvious excitotoxic neuronal cell death, the consequent Müller cell edema seen in Figure 4 could be contributing to the long-term b-wave suppression, probably as a result of compromised potassium siphoning capabilities.[24] Müller cell swelling in response to exogenously applied glutamate recently has been reported in rat retinal segments *ex vivo*.[25] These authors also report no associated excitotoxic neuronal damage unless a very high concentration (3 mM) of glutamate is applied. Thus, the neuronal uptake of glutamate that occurs after the knockdown of GLAST (Fig. 2) probably maintains the extracellular glutamate concentration below excitotoxic levels but above physiological levels necessary for normal signaling between photoreceptors and bipolar cells. These results strongly support and extend the emerging literature, which shows that glial cells play a vital role in mediating glutamatergic neurotransmission.[16,17,22] The direct demonstration of a functional perturbation, as evinced by disruption of the ERG, validates this role in the retina. Conversely, these data also demonstrate that glial cells may exhibit long-term damage (as shown by their swelling) if they are prevented from facilitating normal retinal neurotransmission. Clearly, the association between retinal glia and neurotransmission requires significant consideration when elucidating the fundamental basis of clinically significant pathologic states. Such conditions include ischemic retinal damage after central retinal artery/vein occlusion, which is thought to be associated with elevated extracellular glutamate; in glaucoma, there is evidence for perturbed glutamate homeostasis, as demonstrated by elevated levels of glutamate in Müller cells and in the vitreous humor.[26–31]

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