Rapid Glutamatergic Alterations in the Neural Retina Induced by Retinal Detachment

David M. Sherry¹ and Ellen Townes-Anderson²

PURPOSE. Retinal detachment induces neurochemical changes in the neural retina over a span of days to weeks. However, little information is available on the acute response in the retina to detachment.

METHODS. Distribution of the neurotransmitters glutamate, glycine, and γ-aminobutyric acid (GABA) and the metabolic amino acids aspartate and glutamine was examined immunocytochemically from 5 to 30 minutes and at 3 hours after retinal detachment in a salamander eyecup preparation.

RESULTS. Glutamate showed a rapid depletion from neuronal cell bodies in detached retina, whereas Müller cells, which normally sequester and metabolize glutamate, showed increased immunolabeling for glutamine. Changes occurred exclusively in detached retinal regions of the eyecup. Aspartate, a precursor for glutamate synthesis, also showed decreased labeling in neuronal cell bodies in detached retinal regions, although these changes were not as striking as those observed for glutamate. In contrast, the distributions of the inhibitory amino acid neurotransmitters glycine and GABA were not affected appreciably by acute retinal detachment.

CONCLUSIONS. These results indicate that retinal detachment induces rapid, localized alterations in the glutamatergic system of the neural retina that are consistent with a massive efflux of neuronal glutamate and concomitant alterations in glutamate metabolism. An acute efflux of neuronal glutamate in detached retina could contribute to excitotoxicity and to the initiation of structural alterations and changes in gene expression; it is also consistent with reported neurochemical changes associated with longer term retinal detachment. (Invest Ophthalmol Vis Sci. 2000;41:2779–2790)

Amino acid neurotransmitters affect neuronal survival, growth, and synapse formation in both the developing and adult central nervous system, including retina.¹⁻¹¹ Changes in amino acid transmitters in response to central nervous system insults, therefore, could have profound effects on recovery of function after insult. The excitatory amino acid, glutamate (GLU), and its metabolites, aspartate (ASP) and glutamine (GLN), are of particular interest because insult-induced efflux of neuronal GLU can cause excitotoxic cell death and subsequent functional impairment.¹¹

Detachment of the neural retina from the retinal pigmented epithelium (RPE) induces a variety of changes in retinal neurons and glia. Photoreceptors show disorganization, reduced membrane turnover, eventual loss of outer segments, and, at the synaptic pole, axonal retraction and disruption of ribbon synapses.¹²⁻¹⁶ Second-order neurons respond to detachment by neuritic sprouting and reorganization within the outer plexiform layer.¹²¹⁷ Müller cells show changes in the expression of several cytoskeletal and metabolic proteins.¹⁸⁻¹⁹ In addition, there is a gradual cell loss in the outer and inner retina.¹²⁻²⁰ Finally, neurochemical changes, after detachments lasting 3 hours to several weeks, occur throughout the retina. These changes include alterations in the neurotransmitter and metabolic amino acid contents of virtually all neuronal and glial cells in the neural retina and the cells of the RPE.²¹⁻²² Rapid neurochemical changes also might occur in response to the mechanical trauma and possible injury-induced membrane depolarization caused by retinal detachment. The acute effects of retinal detachment on amino acids in the neural retina, however, are relatively unknown.

We examined the effects of the first 30 minutes of retinal detachment on the distribution of several neurotransmitter and metabolic amino acids in the neural retina using immunocytochemical methods. Experiments were performed using the retina of the tiger salamander because (1) the amino acid transmitter distribution in this species is well described,²³⁻²⁵ and (2) it is a widely used model system for investigating retinal physiology.²⁶⁻³⁰ Retinal detachment induced very rapid and specific changes in the distribution of GLU and the metabolically related amino acids, ASP and GLN. These rapid alterations in the glutamatergic system of the retina could contribute to the subsequent changes in retinal structure, gene expression, and neurochemistry associated with retinal detachment.

METHODS

Animals

Aquatic phase tiger salamanders (Ambystoma tigrinum; 16–23 cm) were maintained in aerated tanks at 4 to 10°C on a 12-hour light:12-hour dark cycle. All animals were light...
adapted and were terminated by decapitation and pithing within ±1 hour of subjective noon. A total of 19 animals were used. All animal procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Preparation of Eyecups
Eyes were removed, and the cornea, lens, and iris were cut away to produce an eyecup. The neural retina was gently teased from the overlying RPE on one half of the eyecup. The neural retina and RPE on the other half of the eyecup were not disturbed, allowing for comparison of intact and detached regions of the same retina. Eyecups were maintained in oxygenated salamander Ringer’s solution for 5, 15, or 30 minutes, or 3 hours at 10°C under room lights. The composition of the serum-free salamander Ringer’s solution was as described previously (in mM): 108 NaCl; 2.5 KCl; 1.0 NaHCO₃; 0.5 Na₂HPO₄; 0.5 MgSO₄; 0.9 CaCl₂; 1.0 Na pyruvate; 24 glucose; 2 HEPES; pH adjusted to 7.7 with NaOH. After incubation, eyecups were fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 to 2 days at 4°C, rinsed in phosphate-buffered saline (PBS), dehydrated, and embedded in epoxy resin.

Antisera
All primary antisera used in these studies were raised in rabbit against amino acid-glutaraldehyde-protein conjugates (Table 1). The specificity of the primary antisera for the appropriate amino acid epitope has been extensively characterized by (1) preadsorption of the primary antiserum with appropriate or inappropriate conjugates, (2) a postembedding specificity test with resin-embedded amino acid-glutaraldehyde-bovine serum albumin conjugates of glutamate, aspartate, γ-aminobutyric acid (GABA), glutamine, glutamic acid (Glu), and glycine.

TABLE 1. Amino Acid Antisera

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<tr>
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<td>Robert E. Marc, PhD†</td>
<td>34,37,38,40</td>
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<tr>
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‡ Signature Immunologics, Salt Lake City, UT.

FIGURE 1. Acute alterations in GLU distribution are localized to detached retinal regions. Low power micrograph of GLU-IR at the border between intact and detached retinal regions of an eyecup incubated for 30 minutes in oxygenated Ringer’s. The transition from normal to altered GLU-IR is abrupt and occurs at the point of detachment (arrows). Intense, specific GLU-IR is localized to neurons and the RPE in the attached retina (ATT; left side). GLU-IR in the detached retinal region (DET; right side) is reduced in both neurons and the RPE. GLU-IR is severely reduced in the photoreceptors and many other neurons of the detached region. Some other neurons, particularly bipolar cells (b) and displaced bipolar cells (db) retain strong GLU-IR. Both plexiform layers in both attached and detached regions show strong GLU-IR. Calibration bar, 50 μm.
tyric acid (GABA), and taurine, and (3) immunoblot tests of crossreactivity with amino acid conjugates. 21,34–40

**Immunocytochemistry**

Pools of endogenous amino acids were visualized in 0.5- to 1-μm semithin sections of resin-embedded tissue using postem-
with 2% to 10% normal goat serum + 0.1% Triton X-100 for 1 to 2 hours. In some experiments the blocking solution was supplemented with 5% bovine serum albumin and 0.5 to 1% fish gelatin. Excess blocking agent was removed, sections were incubated in primary antibody for 16 hours to 2 days at 4°C, rinsed in PBS, and blocked for 30 minutes at room temperature. For immunofluorescence experiments, binding of the primary antiserum was visualized by applying goat anti-rabbit fluorescein diluted 1:30 (Boehringer-Mannheim, Indianapolis, IN) or goat anti-rabbit CY3 diluted 1:400 (Jackson Immunoresearch Laboratories, West Grove, PA) for 45 minutes at room temperature. For ABC experiments, sections were incubated in biotinylated goat anti-rabbit diluted 1:200 (Vector Laboratories, Burlingame, CA) for 45 minutes at room temperature, and binding of the primary antibody was visualized using the Vectastain Elite kit (Vector Laboratories) according to the manufacturer’s instructions, with diaminobenzidine as the chromagen. Both methods yielded similar results and substitution of normal rabbit serum for the rabbit polyclonal primary antisera produced only background immunolabeling, confirming the specificity of the immunocytochemical methods.

**Image Analysis**

To measure detachment-induced changes in GLU and GLN, quantitative analysis of immunoreactivity (IR) was performed using matched pairs of images captured from intact and detached retinal regions within a section. Image pairs from a minimum of three eyecups from different animals were used for all quantitative analyses. Greyscale images (256 gray levels) were captured directly from an Olympus IX70 microscope.
using a Cohu 4915–2000 CCD video camera (Cohu Inc., San Diego, CA), a Scion LG3 digitizing board (Scion Corporation, Frederick, MD), and NIH Image software (Wayne Rasband, National Institutes of Health, Bethesda, MD). Images were captured by setting the camera gain to maximum and averaging frames to reduce noise (32 frames/image for GLU-IR; 16–24 frames/image for GLN-IR). No enhancements of brightness, contrast, or other features were performed. All images were captured at a magnification of $320$ from sections of 0.5–$200\mu m$ thickness labeled with primary antibody diluted 1:1000 and using the goat anti-rabbit fluorescein secondary antibody. The greyscale of all images was linear.

For analysis by cell type, individual retinal cells in the images were identified by their morphologic characteristics and placement in the retina, outlined by hand using the NIH Image freehand selection tool, and the mean gray level was measured. Cell types sampled for analysis of GLU-IR included rod, cone, displaced bipolar, bipolar, horizontal, amacrine, ganglion, and Müller cells. Analysis of GLN-IR was restricted to Müller cells. To assess GLU-IR in the outer and inner plexiform layers (OPL and IPL, respectively), the edges of these layers were outlined using the NIH Image polygonal selection tool, and the mean gray level of the selected layer was measured. Labeling intensities for each cell type or layer in detached retina were expressed as the percent of IR intensity of the same cell type or layer in the intact retinal region of the same section, which was defined as 100%. All statistical analyses of labeling intensities for each cell type or layer in intact versus detached regions was performed on the untransformed gray level data using Student’s two-tailed $t$-test (Statview 4.5 software; Abacus Concepts, Berkeley, CA).

### RESULTS

Immunolabeling for each amino acid was distributed appropriately in intact retinal regions at all time points examined. Retinal detachment, however, induced rapid changes in the immunolabeling patterns of GLU, GLN, and ASP, but did not affect GABA or glycine (GLY). Alterations were restricted to detached retinal regions, indicating that the effects of retinal detachment were highly localized.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/)

**FIGURE 4.** Quantitative analysis of acute retinal detachment-induced changes in GLU-IR intensity in the OPL and IPL. Each panel shows a graphical comparison of GLU-IR in the plexiform layers of detached retinal regions (black circles) expressed as a percentage of GLU-IR in intact retina over time after retinal detachment ($\pm$SEM). The white circles indicate the percentage of GLU-IR in intact retina (defined as 100%) for comparison. There were no statistically significant differences in GLU-IR in either plexiform layer at any time point examined, as determined by analysis of untransformed data. Sample sizes are summarized in Table 2.
Glutamate

In the intact retina, as reported previously for salamander retina, strong GLU-IR was present in most retinal neurons, both plexiform layers and the RPE, whereas rod photoreceptors and Müller cells showed very low GLU-IR levels (Figs. 1, 2A). Retinal detachment caused a rapid decrease in GLU-IR in the detached region that developed progressively from outer retina to inner retina (Figs. 1, 2B through 2D). Decreased GLU-IR was evident in the distal retina by 5 minutes after detachment (Fig. 2B): cones showed a decrease in GLU-IR, whereas GLU-IR in the OPL, IPL, and nuclear layers of the inner retina was not affected. By 15 minutes after retinal detachment changes in inner retina became evident (Fig. 2C). Many neurons in the inner nuclear layer (INL) exhibited decreased GLU-IR, in addition to the cones, which continued to show reduced GLU-IR. Labeling in the OPL, IPL, and ganglion cell layer (GCL) was not altered. Decreased GLU-IR was evident in the GCL of detached regions by 30 minutes after detachment (Fig. 2D). At this time point, reduced GLU-IR became more pronounced in the neurons of the INL and also persisted in the cones, although some subsets of neurons, particularly some bipolar and displaced bipolar cells, continued to show strong GLU-IR. Labeling in the OPL and IPL was not significantly decreased.

Quantitative analysis of GLU-IR confirmed that GLU-IR was specifically depleted in detached retinal regions compared to intact retinal regions and showed that changes in GLU-IR were cell type specific (Fig. 3; sample sizes summarized in Table 2). GLU-IR intensity in cones was significantly decreased in detached retinal regions at all time points. Rods, however, showed no change in GLU-IR, possibly because of the very low GLU-IR levels normally present in these cells even in the intact retina. Bipolar cells showed significantly reduced GLU-IR by 15 minutes after detachment that remained reduced at 30 minutes, although there was clearly heterogeneity in GLU-IR intensities within the bipolar cell population. Displaced bipolar cells with their cell bodies in the ONL, were more homogeneous with respect to GLU-IR intensity and did not show any significant change in GLU-IR intensity at any time point. Quantitative analysis revealed an elevation and a decrease in horizontal cell GLU-IR at the 5- and 15-minute time points, respectively, that was not obvious from qualitative analysis. By 30 minutes after detachment, horizontal cell GLU-IR was comparable in intact and detached retinal regions. GLU-IR in the amacrine cells located in the innermost portion of the INL was significantly reduced in detached retina by 15 minutes and remained depressed at 30 minutes after detachment. A significant reduction of GLU-IR in ganglion cells in the innermost retina was present only at the 30-minute time point. Müller cells showed no significant changes in GLU-IR at any time point. Finally, although GLU-IR did decrease somewhat in both plexiform layers in detached regions over time (Fig. 4), these changes were not significant. These analyses indicate that retinal detachment caused rapid, cell type-specific changes in neuronal GLU levels on a time scale of minutes and that somatic pools were affected more severely than synaptic pools. These results also corroborate the observation that decreased GLU-IR was restricted specifically to detached retinal regions.

Distribution of GLU-IR also was examined in eyecups incubated in oxygenated Ringer’s solution for 3 hours (Fig. 5). Although GLU-IR was reduced in all retinal regions under these conditions, depletion was more pronounced in detached retinal regions than in intact regions, suggesting that GLU in detached retinal regions remains disrupted for a protracted period after the initial insult.

**FIGURE 5.** Altered retinal GLU distribution persists for at least 3 hours after retinal detachment. (A) Although labeling is reduced, GLU-IR in intact retinal regions after a 3-hour incubation resembles the normal distribution of GLU-IR. Cone cells (c) and the neurons of the inner retina show GLU-IR in both plexiform layers. Müller cells (m) continue to show only low levels of GLU-IR, although rods show GLU-IR in their inner segments (+). GLU-IR in the RPE is reduced compared to normal. (B) Reduced GLU-IR persists in detached retinal regions after a 3-hour incubation. Labeling in photoreceptors and most neurons remains reduced compared to intact retinal regions. GLU-IR persists in both plexiform layers of detached retina even after 3 hours. Calibration bar, 20 μm.
Glutamine

GLN is the major metabolite of GLU catabolism in the retina. Most GLU metabolism in the retina occurs in the Müller cells, which avidly sequester extracellular GLU and rapidly metabolize it to GLN via glutamine synthetase. Therefore, detachment-induced changes in retinal GLU might be reflected in alterations of retinal GLN. Retinal detachment induced acute, localized changes in retinal GLN-IR (Figs. 6, 7). In intact retinal regions, strong GLN-IR was present in Müller cell bodies and their apical processes, with the descending processes and endfeet showing very little immunolabeling. Weaker GLN-IR was present in some neuronal cell bodies (Fig. 6A). By 5 minutes after detachment, the apical processes of the Müller cells showed increased GLN-IR. Cone inner segments also showed elevated GLN-IR (Fig. 6B). By 15 minutes after detachment, GLN-IR at the inner limiting membrane was also elevated (Fig. 6C). Elevated GLN-IR was evident throughout the integrity of the Müller cells, including the processes spanning the IPL and the endfeet by 30 minutes after detachment (Fig. 6D).

To determine whether the apparent increase in GLN-IR intensity in the Müller cells of detached retinal regions was due to increased intracellular GLN concentration, the maximal dilution of antiserum that yielded detectable GLN-IR was determined in fluorescently labeled specimens. The dilution of an antiserum required to produce detectable labeling is related to the amount of antigen present. A concentrated antigen can be detected using a very dilute primary antiserum. As antigen concentration decreases, however, the dilution of primary antiserum required to detect the antigen also decreases. Therefore, the maximal dilution of GLN antiserum that produced detectable labeling.

**FIGURE 6.** Acute alteration of retinal GLN distribution in response to retinal detachment. (A) Normal distribution of GLN-IR in intact retina after a 30-minute incubation in oxygenated Ringer’s. Labeling is primarily localized to Müller cell bodies (m) in the INL and their apical processes in the ONL. The descending processes (arrowheads) and endfeet (+) of the Müller cells show only weak GLN-IR. Most neurons in the inner retina show weak GLN-IR although some more intensely labeled neurons are visible in the INL and GCL. Cone cell bodies (c) show weak GLN-IR, but rods (r) show only background labeling levels. The RPE shows moderate labeling. (B) Five minutes after detachment. Increased GLN-IR levels are evident in Müller cell bodies and their apical processes. A slight increase in labeling intensity may be present in Müller cell endfeet (+). Cone inner segments (cis) show a slight increase in GLN-IR, but GLN-IR in most neurons did not appear to be altered. (C) Fifteen minutes after detachment. GLN-IR levels in Müller cells continue to increase and labeling along the inner limiting membrane (ILM) becomes apparent. The descending processes (arrowheads) and endfeet (+) of the Müller cells continue to show elevated labeling. (D) Thirty minutes after detachment. Müller cells show intense labeling throughout their entirety including their descending processes traversing the IPL (arrowheads) and their endfeet (+). Weak labeling persists in cone inner segments (cis) and appears in some rod inner segments (ris). Labeling in the neurons of the inner retina appears unaffected. Calibration bar, 20 μm.
("dilution threshold") was used to compare the relative GLN concentration in the Müller cells of intact and detached retinal regions in eyecups incubated for 30 minutes, when the maximal change in Müller cell GLN labeling was observed. Müller cells in detached retinal regions always showed a higher dilution threshold (1:10,000) than Müller cells from attached regions of the same retina (1:5000), indicating that the GLN concentration in the Müller cells of

**FIGURE 7.** Alterations in GLN distribution are restricted to detached retinal regions. Low-power micrograph of GLN-IR at the border between intact and detached retinal regions of an eyecup incubated for 30 minutes in oxygenated Ringer's. The transition from normal to altered GLN-IR occurs at the point of detachment (large arrow). Labeling is found primarily in Müller cells (m) in both regions; however, the intensity of Müller cell labeling in the intact retinal region (ATT; left) is lower than that in the detached retinal region (DET; right). There is also clear labeling of Müller cell descending processes (arrowheads) and endfeet (small arrows) in the detached region that is not evident in the intact region. Labeling in the RPE is comparable in attached and detached regions. Calibration bar, 50 µm.

**FIGURE 8.** Altered retinal GLN distribution persists for at least 3 hours after retinal detachment. (A) Müller cells (m) dominate the GLN-IR labeling pattern in intact retinal regions after a 3-hour incubation, similar to the normal distribution of GLN-IR. Labeling in the descending processes and endfeet (+) of some Müller cells in intact retina remains weak, although their apical processes show increased labeling. Weak GLN-IR is also present in some neurons, similar to earlier time points. Labeling in the RPE is diminished. (B) Müller cells (m) also dominate the GLN-IR labeling pattern in detached retina after a 3-hour incubation, with persistent elevated labeling of all endfeet (+). Weak labeling persists in some neurons, but labeling of cone cell bodies (c) and their inner segments (arrows) is elevated. These results indicate that changes in retinal GLN induced by retinal detachment persist for several hours after the initial insult. Rod cell bodies (r). Calibration bar, 20 µm.
detached retinal regions was elevated compared with Müller cells in intact retinal regions. Analysis of GLN immunolabeling intensity confirmed these results. Müller cells in detached retinal regions showed a 17% increase in labeling intensity over Müller cells in intact retinal regions (P < 0.0001; n = 67 Müller cells from intact retina; 67 Müller cells from detached retina). Together these analyses confirmed that retinal detachment increased GLN concentration in Müller cells located in detached retinal regions.

The distribution of GLN-IR also was examined in eyecups incubated in oxygenated Ringer’s solution for 3 hours (Fig. 8). Müller cells dominated the GLN-IR pattern in both attached and detached retinal regions at the 3-hour time point. Similar to results at earlier time points, labeling of Müller cell endfeet was more consistently elevated in detached retinal regions than in intact regions even after 3 hours, suggesting that alterations in retinal GLN also persisted for protracted periods after the initial insult.
Aspartate

ASP levels and distribution in the salamander retina are plastic and do not reach a stable distribution until the eyecup has been incubated in oxygenated Ringer’s solution for at least 30 minutes.\(^4^4\) Therefore, analysis of the effects of retinal detachment on ASP was restricted to eyecups incubated for 30 minutes. The distribution of ASP-IR in the intact retina of eyecups incubated for 30 minutes matched those described previously (Fig. 9A).\(^4^4\) ASP-IR was present in cone cell bodies and inner segments, subpopulations of amacrine and bipolar cells in the INL, many cell bodies in the GCL, and both plexiform layers. Retinal detachment caused local alterations in ASP-IR, although the changes were not as striking as those observed for GLU-IR and GLN-IR (Fig. 9B). Fewer neuronal cell bodies showed ASP-IR in detached retinal regions than in intact regions, but ASP-IR persisted in both plexiform layers. Cone cell bodies and inner segments showed reduced labeling, although strong labeling persisted in many bipolar cells and their Landolt’s clubs. Müller cell bodies in detached retinal regions showed increased ASP-IR.

GABA

Distribution of GABA-IR in intact retinal regions was as described previously for salamander retina\(^2^3\) and included labeling of horizontal cells, amacrine cells, displaced amacrine cells, a subset of bipolar cells, and numerous processes in both plexiform layers. The distribution of GABA-IR in detached retinal regions matched that of intact retina and was not altered by retinal detachment at any time point examined (Fig. 10).

Glycine

Immunolabeling for GLY was as described previously for salamander retina\(^2^3\) and included amacrine and displaced amacrine cells, a subset of bipolar cells, and numerous boutons in the IPL. A very sparse plexus of GLY-IR processes was present in the OPL. Weak GLY-IR was noted in the cell bodies and inner segments of cones and in the distal portion of Müller cell bodies in the INL. The distribution of GLY-IR in detached retinal regions was similar to that of intact retina and was not altered by retinal detachment at any time point examined (Fig. 11).

DISCUSSION

Rapid efflux of GLU from retinal neurons is the most likely explanation for the observed decrease in GLU immunolabeling in detached retina. Rapid efflux of GLU from neuronal stores in response to acute insults elsewhere in the central nervous system is well documented.\(^1^1,1^4,4^6\) The increase in Müller cell GLN content is consistent with a massive efflux of GLU from nerve cells. Müller cells are the principal site of GLU uptake in the retina and metabolize GLU to GLN very rapidly via glutamine synthetase.\(^4^1,4^2\) A large efflux of GLU would be predicted to cause increases in GLU uptake and its metabolism to GLN by Müller cells, leading to increased GLN concentration within the Müller cells. Increased Müller cell GLN levels also could arise from reduced release of GLN from the Müller cells back to neurons, and reduced GLN efflux from Müller cell stores could in turn contribute to reduced GLU levels noted in neurons; however, because the onset of GLU depletion preceded the onset of GLN accumulation in detached retina, it seems more likely that changes in GLN are secondary to changes in GLU.

The depletion of GLU in response to retinal detachment differs from the transient depletion of photoreceptor GLU that can be induced by retina preparation methods and then restored by a short “recovery” incubation in oxygenated Ringer’s solution in some species.\(^3^4\) Glutamate depletion in detached retina persisted for at least 3 hours in oxygenated Ringer’s in spite of the fact that these experiments were performed under conditions where glucose and oxygen were available and supported apparently normal GLU metabolism in intact retinal regions. Thus, GLU depletion in these studies was due to retinal detachment and not the eyecup preparation or incubation conditions. The progression of GLU depletion from the photoreceptors to the inner retina is consistent with the site of the initial insult being located at the junction of the RPE and the photoreceptors.

The trigger for GLU efflux in detached retinal regions could be related to spreading depression, a generalized depolarization of neurons and glia associated with mechanical, electrical, chemical, or hypoxic insults that causes massive redistribution of ions and amino acids. Efflux of GLU is a well-
known characteristic of spreading depression. However, spreading depression also can elicit efflux of the inhibitory amino acid transmitters GABA and GLY, which is in contrast to the current report in which GABA and GLY in detached retina were essentially undisturbed. It should be noted, however, that the specific profile of amino acids released in response to insult can differ according to the type of insult. If the changes in the glutamatergic system are triggered by spreading depression, detachment-induced depolarization must be restricted to the detached region of the retina because alterations of GLU and GLN did not extend into intact retinal regions.

A possible molecular mechanism for GLU depletion is depolarization-induced reversal of excitatory amino acid transporters (EAATs). This would result in the preferential efflux of metabolic rather than synaptic pools and thus is consistent with more pronounced depletion of GLU-IR from nuclear layers than synaptic layers in detached retina. However, depolarization of isolated retinal photoreceptors, a condition that is known to cause transmitter release, also can result in reduced somatic glutamate. Some release from synaptic terminals most likely occurs. A recent study in salamander retina identified and localized five different EAATs with high homology to the human EAATs, EAAT1, EAAT2, and EAAT5 (sEAAT1, sEAAT2, sEAAT2B, sEAAT5A, sEAAT5B). The prominent expression of sEAAT1 on photoreceptor cell bodies and EAAT2A by neurons in the inner retina would be consistent with a role for these transporters in mediating acute GLU efflux after retinal detachment. In contrast, the anatomic distribution of EAAT2B in salamander retina is similar to the distribution of bipolar and displaced bipolar cell populations that tended to retain their GLU content after retinal detachment, suggesting that this transporter may be insensitive to detachment injury.

Differences in the regulation of EAATs could partially explain differences in GLU depletion among the various retinal cell types. The role of ASP in the retina appears to be primarily metabolic and acute changes in retinal ASP in response to retinal detachment could be due to several factors, including altered ASP release and/or uptake, altered ASP metabolism associated with changes in GLU, or some combination of those processes. Anoxia or hypoxia can alter ASP levels in retinal neurons. It is unlikely that the detachment-induced decrease in retinal ASP noted in the current studies was due to anoxia or hypoxia because the eyecups were incubated in oxygenated Ringer’s containing both glucose and pyruvate to support aerobic metabolism. Furthermore, anoxia and hypoxia increase ASP levels in specific populations of retinal neurons, particularly photoreceptors, the opposite of what was observed in acutely detached retinal regions.

Previous studies of neurochemical changes in response to retinal detachment have focused on time points beginning at least 1 day after detachment. The current studies indicate that the onset of neurochemical changes in detached retina can occur within 5 minutes of detachment and that disruption of the glutamatergic system of the retina could be one of the earliest changes caused by retinal detachment. Although the acute changes in neuronal GLU noted in these studies persisted for at least 3 hours, these changes may be reversed with time, inasmuch as a study of amino acid signatures associated with retinal detachment showed that neuronal GLU distribution was not disturbed to any great extent 3 days after detachment in vivo. There are also other neurochemical differences between short- and long-term detachment. No increase in GLU was observed in Müller cells over the short term, but was a major feature of long-term detachment, possibly indicating long-term, but not short-term, reduction of glutamine synthetase activity. Furthermore, neuronal GLU and GABA content oscillate synchronously over the long term, whereas during the short term, the GABAergic system appeared unperturbed by retinal detachment. These differences in long- and short-term responses are not inconsistent with one another and suggest that amino acid abnormalities produced by detachment are not static.

An acute efflux of GLU in response to detachment could potentially induce a variety of responses in the retina. Vacuolization and some cell loss in the neural retina have been reported in the first few days after experimental induction of retinal detachment, consistent with limited GLU excitotoxicity. Abnormally high but sublethal amounts of extracellular GLU may also affect cellular metabolism, intracellular signaling pathways, and gene expression. Changes in macromolecular expression, presumably resulting from altered gene expression, are well documented for Müller cells after detachment. Finally, disorganization of photoreceptor terminal ultrastructure, retraction of photoreceptor terminals into the ONL, and extension of horizontal and bipolar cell neurites into the ONL have been reported as soon as 1 day after detachment. GLU may regulate neurite growth in adult as well as developing neurons and is well known for mediating synaptic plasticity in adult neurons (reviewed in Refs. 4 and 6). Future assessment of the functional effects of neurochemical changes associated with retinal detachment should include the rapid alterations observed here in retinal GLU.

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References


