Long-term cellular and regional specificity of the photoreceptor toxin, iodoacetic acid (IAA), in the rabbit retina

LI LIANG,[1] YOSHIAKI KATAGIRI,[2] LUISA M. FRANCO,[2] YASUYUKI YAMAUCHI,[2,3]
VOLKER ENZMANN,[2,4] HENRY J. KAPLAN,[2] AND JULIE H. SANDELL[1]

[1]Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, Massachusetts
[2]Department of Ophthalmology & Visual Sciences, University of Louisville, Louisville, Kentucky
[3]Department of Ophthalmology, Tokyo Medical University Hospital, Tokyo, Japan
[4]Department of Ophthalmology, Inselspital, University of Bern, Bern, Switzerland

Abstract

This study investigated the anatomical consequences of a photoreceptor toxin, iodoacetic acid (IAA), in the rabbit retina. Retinae were examined 2 weeks, 1, 3, and 6 months after systemic IAA injection. The retinae were processed using standard histological methods to assess the gross morphology and topographical distribution of damage, and by immunohistochemistry to examine specific cell populations in the retina. Degeneration was restricted to the photoreceptors and was most common in the ventral retina and visual streak. In damaged regions, the outer nuclear layer was reduced in thickness or eliminated entirely, with a concomitant loss of immunoreactivity for rhodopsin. However, the magnitude of the effect varied between animals with the same IAA dose and survival time, suggesting individual differences in the bioavailability of the toxin. In all eyes, the inner retina remained intact, as judged by the thickness of the inner nuclear layer, and by the pattern of immunoreactivity for protein kinase C-α (rod bipolar cells) and calbindin D-28 (horizontal cells). Müller cell stalks became immunoreactive for glial fibrillary acidic protein (GFAP) even in IAA-treated retinae that had no signs of cell loss, indicating a response of the retina to the toxin. However, no marked hypertrophy or proliferation of Müller cells was observed with either GFAP or vimentin immunohistochemistry. Thus the selective, long lasting damage to the photoreceptors produced by this toxin did not lead to a reorganization of the surviving cells, at least with survival as long as 6 months, in contrast to the remodeling of the inner retina that is observed in inherited retinal degenerations such as retinitis pigmentosa and retinal injuries such as retinal detachment.

Keywords: Retina, Retinal degeneration, Bipolar cells, Retinal injury, Retinitis pigmentosa

Introduction

Inherited retinal degeneration is a relatively common cause of blindness and the most common variety is retinitis pigmentosa (RP), which occurs in about 1 in 3,500 live births (Pagon, 1988). RP is characterized by the onset of night blindness in adolescence and young adulthood, followed by peripheral visual field loss, and finally the loss of central vision. The blindness in RP is caused by the degeneration of the photoreceptors, while the number of cells in the inner retina is fairly well preserved (Santos et al., 1997; Humayun et al., 1999). Although the inner retina survives, it undergoes progressive remodeling, including the migration of neurons and glia and the elaboration of new neurites and synapses by the surviving cells (Milam et al., 1998; Fariss et al., 2000; Marc et al., 2003). Similar remodeling occurs in rodent animal models of

RP (Strettoi & Pignatelli, 2000; Gargini et al., 2007). There is evidence for retinal remodeling in acute retinal injury as well. For example, retinal detachment causes photoreceptor degeneration, kainic acid kills neurons in the inner retina and in both cases the surviving cells sprout neurites and undergo synaptic rearrangement (Peichl & Bolz, 1984; Fisher & Lewis, 2003). In contrast, remodeling of the neurons of the inner retina does not seem to occur in response to the photoreceptor degeneration caused by intense light exposure (Noell et al., 1966; O'Steen et al., 1972; Aonuma et al., 1999), although the inner retina does degenerate if the light intensity is extremely high (McKechnie & Foulds, 1980). Likewise, when a chemical toxin such as N-methyl-N-nitrosourea (MNU) kills photoreceptors, the inner retina remains intact (Tyler & Burns, 1991; Nakajima et al., 1996).

Müller glial cells may be even more sensitive than neurons to retinal damage or stress (reviewed by Bringmann et al., 2006). They show signs of activation, including hypertrophy, and increased expression of intermediate filament proteins, in response to retinal detachment (reviewed by Fisher & Lewis, 2003), kainate neurotoxicity (Chang et al., 2007), MNU toxicity (Tyler & Burns,

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Address correspondence and reprint requests to: Julie H. Sandell, Department of Anatomy & Neurobiology, Room R-1014, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118. E-mail: jsandell@bu.edu

1991), retinitis pigmentosa (Fariss et al., 2000), and normal aging (DiLoreto et al., 1995). Moreover, Müller cell activation can have far-reaching effects in the retina, possibly inducing neuronal degeneration at sites that are at a distance from the primary injury (Francke et al., 2005).

The present study investigated the effects of retinal injury produced by the photoreceptor toxin, iodoacetic acid (IAA), in the rabbit. The toxicity of IAA is based on the inhibition of glyceraldehyde-3-phosphate dehydrogenase (Winkler et al., 2003), causing a lowering of the metabolic energy production and consequent loss of function and death of the photoreceptors. Since the effects of IAA were first described (Noell, 1951), retinae altered by this toxin have been considered as a possible model for retinitis pigmentosa (Noell, 1953; Humayun et al., 1995) because of the predilection of the toxin for photoreceptors. Within 3–6 h of systemic injection of IAA in the rabbit, degenerative changes were observed in the photoreceptor inner and outer segments, including swollen mitochondria, and disorganized and excessive disk membranes, and by 2–3 days most photoreceptors had broken-down (Orzalesi et al., 1970). The inner segments disappeared prior to the outer segments, but by 10–15 days, virtually all photoreceptors were gone, and the space formerly occupied by their outer segments now contained the apical edge of the Müller cells.

The present study revisited the use of IAA to kill photoreceptors with several goals: to examine the topography of IAA toxicity in a systematic way in order to develop a better understanding of how a blood borne toxin affects photoreceptors in the rabbit, to examine specific cell populations in the inner retina at survival times as long as 6 months to determine whether there is remodeling of the remaining cells in this photoreceptor degeneration model, and to place the structural effects of this toxin in the context of other forms of induced or inherited retinal degeneration.

Materials and methods

Animals

Eighteen adult Dutch-belted rabbits (2–2.5 kg) were used in this study and were treated in accordance with the Society for Neuroscience statement for the use of animals in research and following approval by the University of Louisville IACUC. The animals received an intravenous injection of 20 mg/kg body weight of sterile IAA (Sigma, St. Louis, MO); a dose described in the literature as effective in creating photoreceptor degeneration but also causing a relatively high mortality of 20% (Orzalesi et al., 1970). At the conclusion of the study, the animals were euthanized with Beuthanasia-D (100 mg/kg pentobarbital, i.v.); eyes were enucleated and processed histologically. Twenty-five retinae were available for anatomical studies following IAA administration, including six with 14-day survival, six with 1-month survival, five with 3-month survival, and six with 6-month survival, as well as two healthy control retinae.

Fixation

Eyes were fixed by immersion in 4% paraformaldehyde in phosphate (PO_4) buffer (0.1 M, pH 7.4) and stored in fixative at 4°C for at least 1 week. The anterior segment, lens and vitreous were then removed, and specimens containing retina, RPE, and choroid were prepared. Although every effort was made to prevent detachment of the retina from the RPE during histological processing, this often occurred in cases in which the photoreceptors were damaged because of the treatment. IAA is also known to produce separation of the RPE from the retina within a matter of hours (Ashburn et al., 1980). Although a high glutaraldehyde fixative might have maintained this attachment, glutaraldehyde was incompatible with the immunohistochemical studies performed on these eyes.

Plastic sections

A strip of retina about 3 mm wide that extended from the dorsal to the ventral margin of the eye was divided into three or four pieces. Each piece was notched to preserve identification of its dorsal edge. The pieces were dehydrated in ascending ethanol concentrations, infiltrated, and embedded in JB-4 Plus resin (Ted Pella, Redding, CA). Tissue was oriented within the block so that vertical sections could be obtained along the dorsal to ventral axis and the tissue blocks were trimmed to preserve this orientation information. Sections 4 microns thick were cut on a rotary microtome (Shandon Lipshaw, Pittsburgh, PA), mounted on slides, dried, and stained with 1% cresyl violet. Sections were examined at 40 or 100× using a Zeiss Axioskop microscope (Zeiss, Thornwood, NY).

Nuclear profile counts and statistics

To determine how the IAA treatment affected the sensory retina, the number of rows of nuclei in vertical sections of the outer and inner nuclear layer was counted in four locations from each retina: dorsal (2.8 mm dorsal to the center of the medullary rays), rays (center of the medullary rays), visual streak (at the peak of the visual streak, as judged by the density of nuclei in the retinal ganglion cell layer), and ventral (2.8 mm ventral to the location where the visual streak count was made). Ten adjacent counts were made at each of these locations in each retina without knowledge of the treatment condition (Fig. 1). The mean number of rows of nuclear profiles was calculated for each retina in each location. Because there was considerable anatomical variability from retina to retina, we did not group the retinae by survival time for the statistical analysis. Instead, the mean counts from all retinae were analyzed by location (dorsal, rays, streak, or ventral) using one-way ANOVA, followed by post-hoc *t*-tests to determine whether the number of rows of nuclei counted in a treated retina at a given location was significantly reduced compared to the count in the same location in the control retinae. Bonferroni or Dunn corrections were used for these multiple comparisons, with a *P* value of <0.05 taken as indicating a difference from control.

Immunohistochemistry

Pieces of retina adjacent to those used for the nuclear counts were rinsed in PBS buffer (0.1 M, pH 7.4), infiltrated with ascending sucrose concentrations (10%, 20%, 30%), and mounted in OCT embedding compound for cryostat sections. Sections 16 microns thick were thaw-mounted onto gelatin-coated slides and stored at −80°C until further processing. Primary antisera included polyclonal anti-protein kinase C-α isoform to label rod bipolar cells (Cat. #P4334, Sigma, St. Louis, MO; diluted 1:40K), monoclonal anti-opsin to label rod photoreceptors (Cat. #O4886, clone RET-P1, Sigma, St. Louis, MO; diluted 1:64K), monoclonal anti-calbindin D-28K (Cat. #C9848, clone CB-955, Sigma, St. Louis, MO; diluted 1:4K) to label horizontal cells, monoclonal anti-vimentin (Cat. #V6630, Clone V9, Sigma, St. Louis MO, diluted
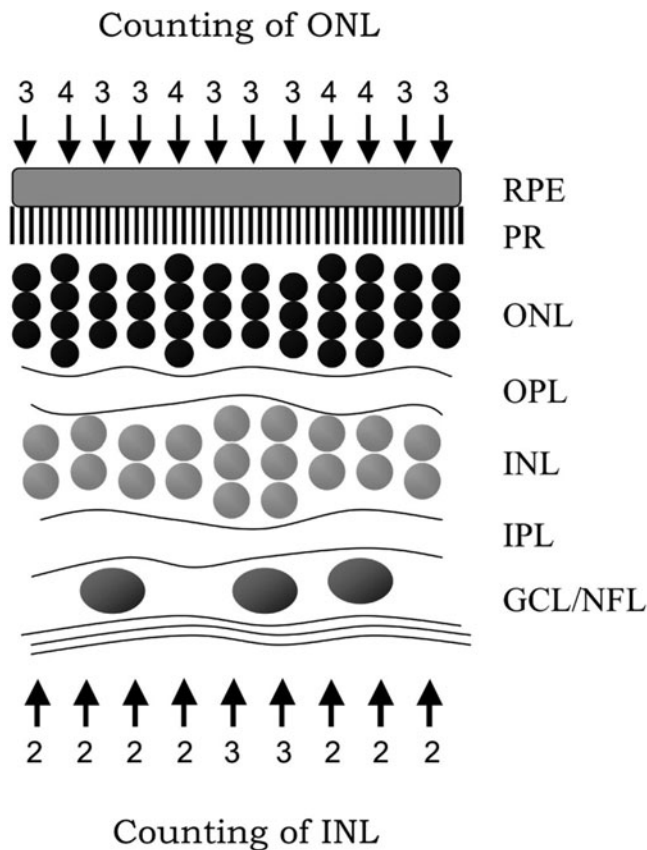
Counting of ONL

3 4 3 3 4 3 3 3 4 4 3 3

RPE
PR
ONL
OPL
INL
IPL
GCL/NFL

2 2 2 2 3 3 2 2 2

Counting of INL

Fig. 1. Schematic diagram showing the retina and sample counts of cell rows in the outer nuclear layer (ONL) and inner nuclear layer (INL). Abbreviations: RPE: retinal pigment epithelium, PR: photoreceptor inner and outer segments, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL/NFL: ganglion cell layer/nerve fiber layer.

1:32K), and polyclonal anti-GFAP (Cat. #Z0334, DAKO, Carpinteria, CA, diluted 1:16K–1:64K) to label filaments in Müller cells.

For anti-opsin, -calbindin, -vimentin, and -GFAP staining, sections were processed using standard procedures as recommended by the Vectastain ABC-Elite kit for mouse or rabbit IgG (Vector Laboratories, Burlingame, CA). In brief, sections were thawed at 37°C, rinsed in PBS buffer, and blocked in 1.5% normal horse serum for 4 h at room temperature. Sections were then incubated in primary antiserum overnight, rinsed in buffer, incubated in biotinylated secondary antiserum for 1 h, followed by avidin-biotin reagent for 1 h. Labeling was visualized with 3, 3′ diaminobezidene and H_2O_2 (DAB kit, Vector Laboratories, Burlingame, CA). For PKC-α and calbindin D-28 fluorescent double labeling, sections were blocked in 5% normal donkey serum at room temperature overnight. Sections were then incubated in a mixture of both primary antibodies at room temperature for 4 h (anti PKC-α at 1:40K, anti-calbindin D-28K at 1:4K). The sections were rinsed in PBS buffer three times for 10 min each, blocked with 5% donkey serum for 1 h, and then incubated in diluted fluorescent secondary antibodies for 45 min at room temperature. The secondary antibodies were rhodamine-conjugated anti-mouse IgG (Cat. #715-025-150, Jackson Immunoresearch, West Grove, PA, 1:400) and

fluorescein-conjugated F (ab') two fragment anti-rabbit IgG (Cat. #711-096-152, Jackson Immunoresearch, West Grove, PA, 1:400). Sections were then rinsed in PBS buffer and coverslipped with polyvinyl alcohol mounting medium with DABCO (Sigma, St. Louis, MO). Sections with fluorescent labeling were examined using a Zeiss LSM 510 confocal microscope. Control sections in which the primary antiserum was omitted were processed simultaneously with sections labeled with each antiserum. No specific labeling was observed in these control sections.

Image processing

For the figures showing the effect of the toxin on retinal histology in plastic sections and for the peroxidative immunohistochemistry, light micrographs on conventional film were scanned, digitized and processed using Adobe Photoshop (Adobe Systems, Inc., San Jose, CA) to improve brightness and contrast, and to remove artifact outside of the sections themselves. The fluorescence images of double label immunohistochemistry obtained by confocal microscopy were processed using Zeiss LSM Image browser version 3.5.0.223 (http://www.zeiss.com).

Results

Nuclear counts

The photoreceptors in the visual streak and ventral region of the retina were the most vulnerable to the effects of systemic IAA administration (Table 1). In the ventral retina, 43% of the eyes exhibited a significant reduction in ONL thickness, compared to 35% in the visual streak, 6% in the medullary rays, and 26% in the dorsal retina. The retina in the far periphery (both dorsal and ventral) appeared to be spared, even in cases in which photoreceptors were completely lost elsewhere in the retina. The shaded boxes in Table 1 indicate locations with a significant reduction in the rows of nuclei in the ONL in the treated retinae compared to control ($P < 0.05$). Fellow eyes that came from the same animal are indicated by a box around the eye numbers. It is obvious from Table 1 that there was considerable variability in the magnitude of the cell loss from animal to animal at the same survival interval. In a few cases, the effects were even different in the two eyes of a single animal despite intravenous injection of the IAA (compare #9 and 10). In sharp contrast, we did not detect any significant change in the thickness of the INL in any retina compared to control, even in cases in which the ONL was reduced in thickness or destroyed.

Plastic sections

The appearance of the retina in the plastic sections used for the nuclear profile counts is shown in Figs. 2–5. The retina shown in Fig. 2 (eye #3) was severely affected when examined 14 days after IAA administration. The ONL had virtually disappeared at all locations except the far periphery. The absence of inner and outer segments meant that the RPE/choroid readily detached from the retina and it is missing from some panels. The remaining retinal layers look qualitatively normal, however, and the INL, IPL, and GCL are readily discernible in the medullary rays (Fig. 2A), visual streak (Fig. 2B), and ventral retina (Fig. 2C).

Fig. 3 illustrates the effects of IAA in two different animals 1 month after IAA administration. In the retina shown in the top panels (eye #9), the thickness of the ONL was significantly re-

Table 1. *The horizontal rows of nuclei in the outer nuclear layer, measured at four different locations in each retina, at different time points after intravenous iodoacetic acid (IAA) injection. In six rabbits, both eyes were available for analysis. Those pairs of eyes are indicated by boxes around the eye numbers (e.g., #6 and #7 came from the same rabbit). All other eyes were single eyes from different animals. Shading indicates values significantly less than control (P < 0.05, following Bonferroni or Dunn correction for multiple post-hoc comparisons)*

Eye #	Treatment	Dorsal rows	Rays rows	Streak rows	Ventral rows
1	0 (control)	4.3	3.1	4.9	3.7
2	0 (control)	n/a	3.5	4.8	4.4
3	14 days IAA	0.2	0	0	0
4	14 days IAA	3.9	3.4	0	2.5
5	14 days IAA	3.8	2.8	5.3	4.7
6	14 days IAA	3.8	4.3	5.6	3.5
7	14 days IAA	3.7	3.9	5.8	4.7
8	14 days IAA	5.4	4.1	6.1	4.6
9	30 days IAA	0.2	2	0	0.2
10	30 days IAA	3.7	3.5	5.2	4.5
11	30 days IAA	3.7	3.6	5.5	0.5
12	30 days IAA	4.1	3	5.4	0.7
13	30 days IAA	4.2	3.9	2.4	0.6
14	30 days IAA	4	3.6	6.1	4.8
15	90 days IAA	3.6	2.7	5.3	4.2
16	90 days IAA	3.9	2.7	5.2	4.7
17	90 days IAA	0	0	0	0
18	90 days IAA	3.5	3.9	5.2	4.4
19	90 days IAA	2.9	3.1	4.4	1.6
20	180 days IAA	3.7	2.6	4.2	4.1
21	180 days IAA	3.9	4	3.7	3.9
22	180 days IAA	3.7	2.9	5	4.4
23	180 days IAA	3.8	3.7	5.1	4
24	180 days IAA	1.2	2.7	3	0.5
25	180 days IAA	1.7	2.7	3.2	2

duced in the medullary rays (Fig. 3A), and was missing in the visual streak and ventral retina (Figs. 3B, 3C). In the retina shown in the bottom panels (eye #11), the thickness of the ONL was normal in most locations (e.g., medullary rays Fig. 3D, visual streak Fig. 3E), but was virtually absent from the ventral retina (Fig. 3F).

With a survival time of 6 months after administration of IAA, the thickness of the ONL ranged from a single layer of nuclei (eye #24, Figs. 4A–4D) to relative preservation of the ONL at all locations (eye #23, Figs. 4E–4H). The appearance of the retina in these locations in a control animal (eye #1) is shown for comparison in Figs. 4I–4L. Note that the effect of the toxin on the ONL was variable at all survival times, despite intravenous administration of a constant dose to all animals. Nevertheless, the toxin was consistently selective for photoreceptors.

Immunohistochemistry

Immunolabeling with the monoclonal antibody RET-P1, which is specific for photoreceptors (Barnstable, 1980), was restricted to the surviving cells in the ONL in all cases. In retinae, in which damage to the ONL was localized (most commonly to the ventral retina), we observed a normal pattern of RET-P1 labeling of photoreceptors both dorsal and ventral to the damaged area. In regions in which the ONL was eliminated, the labeling was absent. This is illustrated in Fig. 5A, which is a photomontage of RET-P1 labeling in the retina of a 14-day IAA animal (eye #4). The montage extends from normal retina in the medullary rays on the left, to retina in the visual streak on the right. The outer and inner segments of the photoreceptors are heavily labeled, and the cell bodies in the ONL are lightly labeled in the normal part of the retina, but the photoreceptor labeling gradually disappears as the ONL disappears. The inset in Fig. 5A shows the ONL/OPL border at high magnification. In some animal models of retinal degeneration, such as the P23H transgenic mouse, translocation of rhodopsin immunoreactivity to the OPL has been reported (Roof et al., 1994). No such translocation was seen in retinae damaged by IAA (inset), and translocation is absent in the rd mouse and the RCS rat (Roof et al., 1994). A montage of sections from the same retina labeled for calbindin D-28, a horizontal cell marker (Lyser et al., 1994), is shown in Fig. 5B. The horizontal cell labeling (arrows) appears to be unperturbed by IAA treatment, even when the entire ONL is missing.

Rod bipolar cells also survive after destruction of the photoreceptors by IAA. Figs. 6A and 6B show double labeling for PKC-α, a rod bipolar cell marker (Martin & Grünert, 1992) in green, and the horizontal cell marker, calbindin D-28 in red, in eye #13, 1 month after IAA administration. The section in Fig. 6A comes from an intact part of this retina and Fig. 6B comes from a damaged region of the same retina. In the damaged part of the retina, the rod bipolar cells and horizontal cells look normal, although the outer nuclear layer is missing. Abnormal neurites from horizontal cells or rod bipolar cells were not detected at any time point, although deafferentation of these cells may well cause changes that are beyond the resolution of the methods used in this study.

Fig. 7 shows immunolabeling for two markers for retinal glia: glial fibrillary acidic protein (GFAP) and vimentin. In the control retina (Fig. 7A), GFAP labeling was confined to the nerve fiber layer (arrows), most likely in astrocytes as described by others (e.g. Schnitzer, 1985). In retinae from animals treated with IAA, GFAP immunoreactivity was detected in the stalks of Müller cells (arrowheads), even in retinae that otherwise appear completely normal (Fig. 7B). In retinae with IAA-induced photoreceptor loss, the GFAP labeling of the Müller cell stalks was intense in both the damaged and undamaged regions of the retina (e.g., Figs. 7C and 7D). Vimentin immunoreactivity labels Müller cell stalks in normal retina (Robinson & Dreher, 1990) as shown in Fig. 7E, and labels them in retina from IAA-treated animals, as shown in Fig. 7F. Although the morphology of the labeled Müller cell stalks remained normal, even in regions with severe damage to the outer retina, the labeling in the stalks in the damaged retina appears to be more intense, and the stalks appear a bit thicker than in the normal retina, which may indicate a mild hypertrophy of the cells. However, no glial scars were observed with either GFAP or vimentin labeling in any animals, including those with the longest survival interval in this study, 6 months. We attempted to detect Müller cell proliferation using antibodies to Ki67, which are known to label Müller cells that proliferate in response to injury (Kohno et al., 2006). Technical issues (rabbit on rabbit immunohistochemistry) produced relatively high background staining, but no Ki67-labeled cells were observed (data not shown).
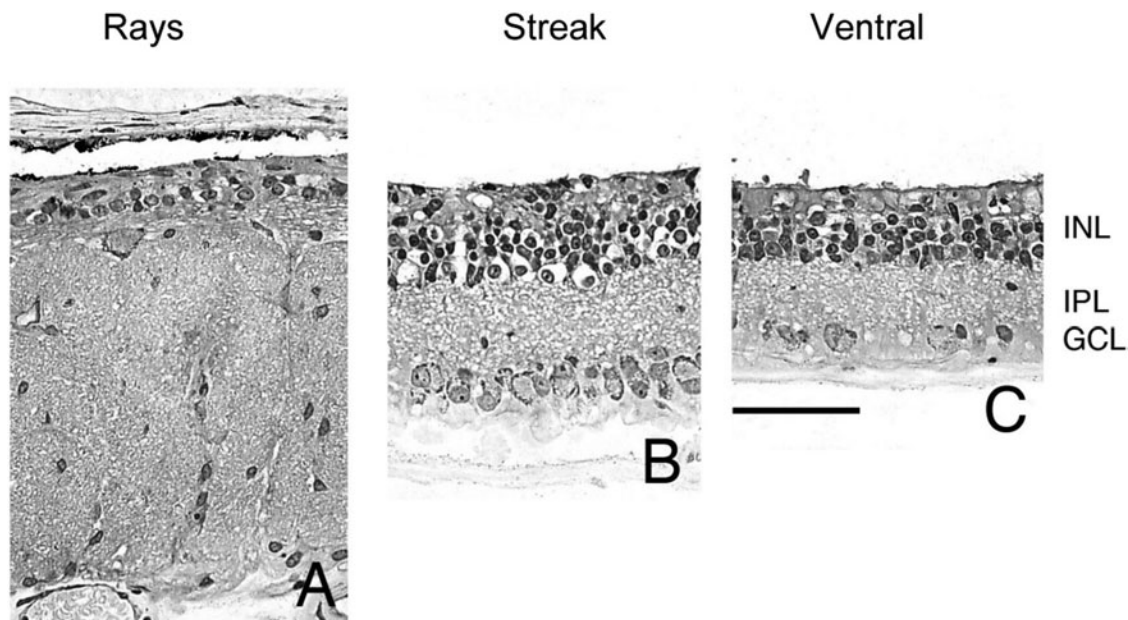
Fig. 2. Sections of retina #3, 14 days after systemic IAA administration. The outer nuclear layer is virtually absent in the medullary rays (**A**), visual streak (**B**) and 2.8 mm ventral to the visual streak (**C**). The inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL) look normal. Scale bar = 50 microns and applies to all panels.

Discussion

Photoreceptor degeneration is involved in the pathogenesis of many eye disorders and can cause severe visual handicap, but so far, specific treatment is lacking. Therefore, experimental models of photoreceptor degeneration have been the subject of much research. Since the first demonstration of the retinotoxic effect of IAA (Noell, 1951), histological and electrophysiological evidence has shown that the toxin acts primarily on the photoreceptors (Noell, 1953; Lasansky & De Robertis, 1959; Humayun et al., 1995). The present study extended these observations by determining the regional distribution of photoreceptor damage, and by examining the sparing of specific cell populations in the inner retina. The major findings are a predilection for damage in the ventral retina, which we hypothesize, is related to the blood supply of the eye and mechanism of action of the toxin, and the sparing of the inner retina, with little evidence of remodeling of neurons or glia.

Regional variation

The extent of the damage to the outer retina was clearly different at different locations, with the most damage in the area ventral to the visual streak. The explanation for this regional vulnerability is not obvious, although two factors should be considered: the distribution of photoreceptors and the pattern of blood vessels. Although IAA is thought to have a predilection for damaging rods (Noell, 1953; Orzalesi et al., 1970), the visual streak, with its many cones (Juliusson et al., 1994) was by no means spared. The rod population in the rabbit retina is actually densest just dorsal to the visual streak (Famiglietti & Sharpe, 1995), yet this region was not the most severely damaged. Moreover, in areas of severe damage the entire ONL was missing, so clearly the toxin can kill both rods and cones.

We also considered whether the regional effect of the toxin could be related to the pattern of blood vessels, since IAA was delivered through the systemic circulation. The vasculature in the rabbit retina has a restricted distribution, with intrinsic blood vessels confined to the medullary rays, a horizontal band of myelinated retinal ganglion cell axons that converge on the optic disk. Since IAA was delivered in the systemic circulation, one might expect it to have the greatest effect on the retina in the medullary rays, which is in closest proximity to these blood vessels, but this was not the case. Moreover, the photoreceptors in the far periphery near the ora serrata did not degenerate, a result that confirms earlier observations in both rabbit and cat retina (Noell, 1953). Taken together, these findings suggest that the relationship of the retina to the choroidal vasculature might be an important factor in the regional variation in IAA toxicity, since choroidal blood flow is highest in the rabbit retina in the area of the streak, and in the far periphery (Nork et al., 2006). The rate of choroidal blood flow in the region ventral to the streak is relatively low. Perhaps proximity of the retina to rapid circulation might provide some protection from the toxin, rather than increased exposure. The only study to examine IAA levels in retinal tissue and blood found a 50% reduction in ^{14}C levels in the blood 3 h after a systemic ^{14}C-IAA injection, but a much slower clearance rate in the retina (Orzalesi et al., 1970). More than 90% of the original level was still present in the retina after 5 h, the longest interval at which the retina was assayed. The study of IAA clearance used the entire retina, but the slower breakdown of IAA in the retina compared to the blood also suggests that retina that is closest to rapid blood flow might be somewhat less vulnerable, compared to retina juxtaposed to slow flow. Investigation of the vasculature per se was beyond the scope of the present study, but Noell reported that IAA did cause an apparent narrowing of the retinal blood vessels in the cat and monkey and a reduced width of the choriocapillaries in the rabbit retina (Noell, 1953). Finally,
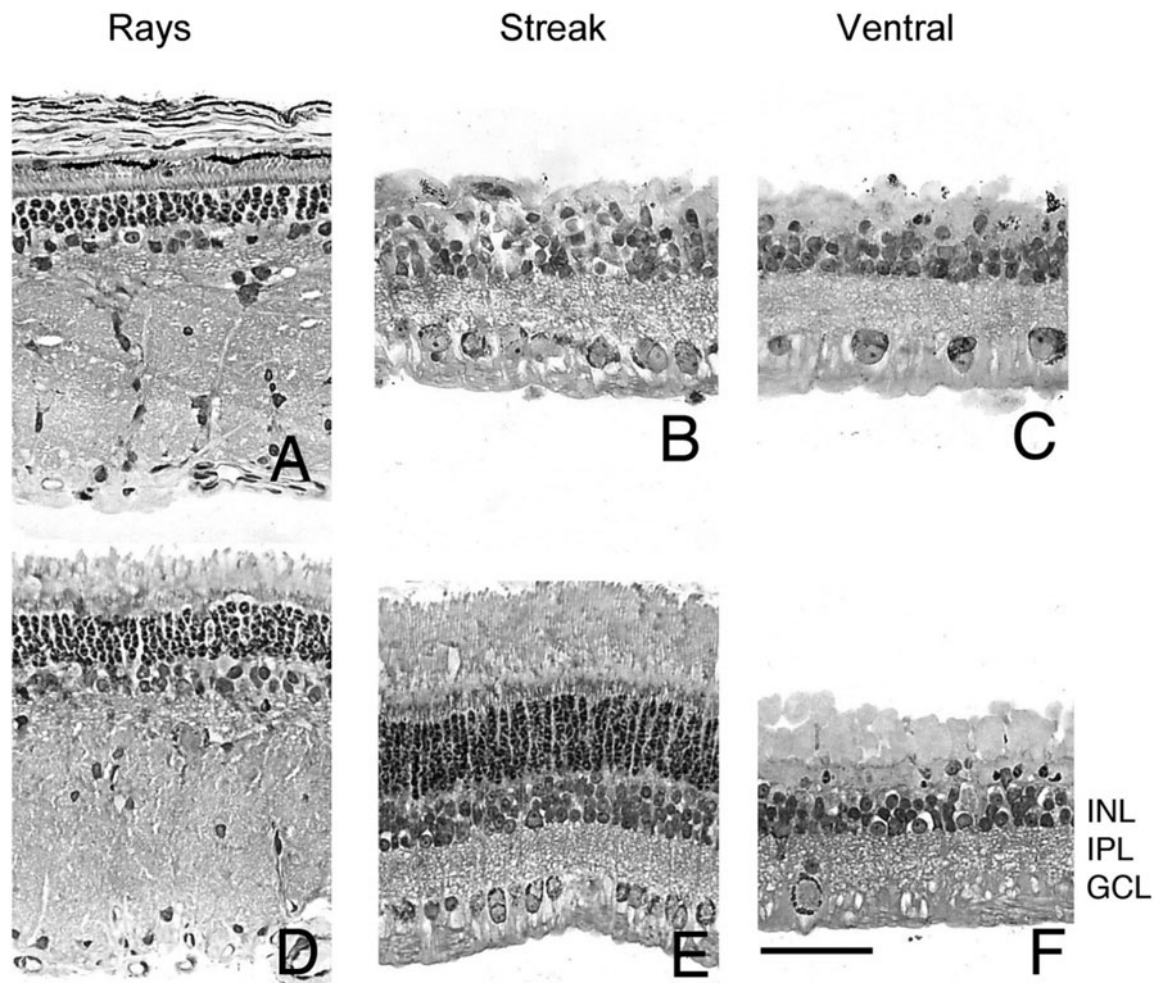
Rays   Streak   Ventral

Fig. 3. Sections of retina #9 (panels **A–C**) and #11 (panels **D–F**), both 1 month after systemic IAA administration. Panels A and D show the retina in the medullary rays, panels B and E show the retina in the visual streak, and panels C and F show the retina 2.8 mm ventral to the streak. In #9 the ONL was reduced to one cell or less in thickness in all locations except the medullary rays. In #11 the only damage was seen in the ventral retina (panel F). Scale bar = 50 microns and applies to all panels. Abbreviations: INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer.

proximity to circulating blood is also related to the availability of oxygen, and oxygen tension within the retina has been implicated in the pathogenesis of various forms of retinal degeneration in humans and animal models (Yu & Cringle, 2005). Since IAA is an inhibitor of anaerobic glycolysis, the fact that the ventral rabbit retina is far from intrinsic blood vessels *and* juxtaposed to choroidal vasculature with low flow may make this region most vulnerable to the toxin, if the ventral retina is more dependent on this metabolic pathway for its energy requirements.

Sparing of the inner retina

Experimental paradigms that destroy photoreceptors have a range of consequences, from little damage to the inner retina, to significant remodeling of neurons and/or glial cells. Exposure to IAA induced GFAP levels in Müller cells that were detectable by immunohistochemistry, even in retinae that showed no other signs of exposure to the toxin. No quantitative or qualitative changes were detected in the inner retina, even when the outer retina was destroyed. Of the various models of photoreceptor degeneration,

these effects may be most similar to those observed when albino animals are exposed to intense or continuous light. In that paradigm, the photoreceptors degenerate by apoptosis (Aonuma et al., 1999; Wasowicz et al., 2002), but cells survive in the inner retina (Seiler et al., 2000), and at least one major inner retinal cell population, the GABA-ergic amacrine cells, retain their normal morphology and distribution (Wasowicz et al., 2002). Likewise, when the retina is exposed to the toxin N-methyl-N-nitrosourea, the photoreceptors degenerate and the Müller glia show signs of activation (Nakajima et al., 1996) but the inner nuclear layers remains morphologically intact.

On the other hand, there are conditions that cause degeneration of subsets of retinal neurons and subsequent remodeling of the remaining cells. Some of these include inherited conditions such as RP and acute insults like retinal detachment or exposure to kainic acid. In human RP and many rodent RP models, the first changes are subtle alterations in neuronal structure, sometimes translocation of the photopigment within the photoreceptors, photoreceptor apoptosis, large-scale reorganization of surviving neurons, and massive involvement of glial cells (Roof et al., 1994; Li et al.,
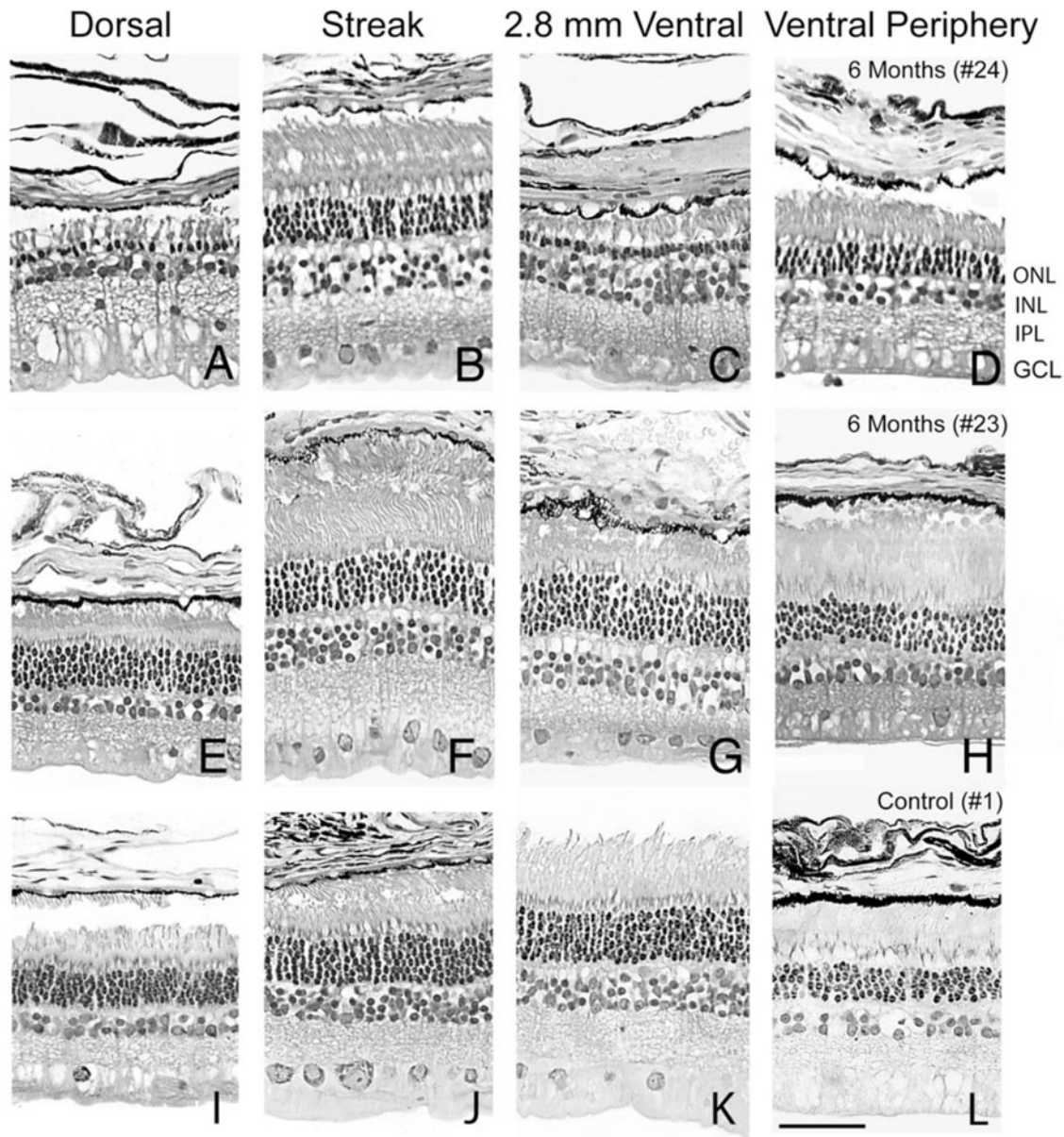
Fig. 4. Sections of retina from four locations in #24 (panels **A–D**) and #23 (panels **E–H**), both 6 months after systemic IAA administration, as well as control retina #1 (panels I–L). In #24 the ONL was reduced to a single layer of nuclei in the dorsal retina and ventral to the visual streak, although the ONL resumed a normal thickness even further ventral (D). In contrast, the ONL in #23 appeared undamaged at all locations. Scale bar = 50 microns and applies to all panels. Abbreviations: ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer.

1995; Milam et al., 1998; Strettoi & Pignatelli, 2000; Marc et al., 2003). However, genetic mutations are present from conception and studies of the rd mouse indicate that the mutation also affects the initial development of the photoreceptors, long before degeneration ensues (Strettoi & Pignatelli, 2000). The mutation presumably continues to act over the lifetime of the individual, and this time course is quite different from a toxin that is administered to an adult animal in a single dose.

However, it cannot be true that time course alone predicts remodeling. Although RP has a long time course and leads to remodeling, while IAA toxicity is quick and remodeling is minimal, there are counter examples. Retinal detachment is an acute insult that produces photoreceptor death in the adult, yet produces

a vigorous glial cell response, and the surviving neurons react by sprouting neurites (reviewed by Fisher & Lewis, 2003). Neurite sprouting is also observed when kainic acid is used to kill inner retinal neurons, rather than photoreceptors. Horizontal cells exposed to a sublethal dose of kainic acid retract their processes in the OPL and sprout abnormal neurites into the deeper layers of the retina, even though the photoreceptors survive (Peichl & Bolz, 1984). Thus, in many, but not all situations in which retinal neurons survive the loss of some of their inputs, they respond by sprouting.

It may be that different results would be observed with different doses of IAA, or a different route of administration. However, despite the systemic administration the effect of the toxin was not
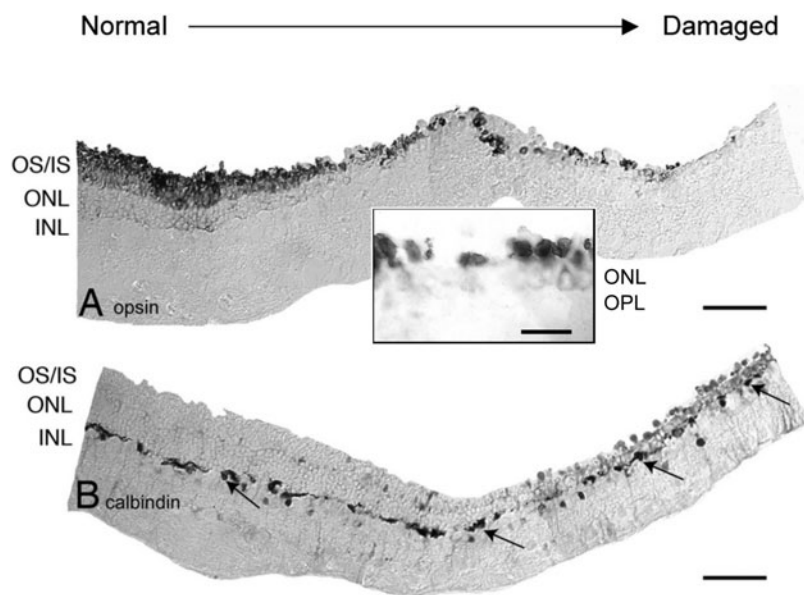
Fig. 5. Sections labeled for specific cell populations. Fig. 5A shows a photomontage of sections from retina #4, 14 days after IAA administration, labeled for opsin with the RET-P1 antibody. Outer and inner segments (OS/IS) of photoreceptors are heavily labeled and the outer nuclear layer (ONL) is more lightly labeled. The montage demonstrates normal retina near the medullary rays (on the left side of image) and shows a progressive loss of the outer retina as one moves ventrally (on the right side of image). Scale bar = 50 microns. The inset in 5A is a high magnification view of the ONL/OPL border in the zone of moderate damage (1–3 cell layers remain in the ONL), labeled for opsin. There is no sign that photopigment has migrated into the OPL. Scale bar for inset = 15 microns. Fig. 5B shows a similar photomontage from the same retina labeled with anti-calbindin D-28 antibody to demonstrate horizontal cells and their lateral processes. The horizontal cells lie near the middle of the retina in the intact region (left side) and come to lie near the retina surface as the ONL disappears (right side). Several horizontal cells are indicated by arrows. Scale bar = 50 microns. Abbreviations: OS/IS: inner and outer segments, ONL: outer nuclear layer, INL: inner nuclear layer.

uniform across the retina, so one would expect that in the transition region between complete destruction of the photoreceptors and complete survival, conditions might have been appropriate to induce neurite sprouting of surviving cells, as is seen with gradients of kainic acid effects. However, this was not observed in the present study. Moreover, it is unlikely that such neurites were not detected for technical reasons since we do detect abnormal neurites in RP retinae, as reported by others, when using the same antibodies and visualization procedures employed for the IAA studies (Liang et al., 2007). Together these findings suggest that something about the mechanism of photoreceptor degeneration is distinct in the case of IAA, such that sprouting is not stimulated, or is inhibited, when photoreceptors are eliminated by this method.

Utility of the IAA model

Although IAA-induced photoreceptor degeneration was originally described as a possible model for retinitis pigmentosa, it clearly has limitations. Foremost is the absence of remodeling in the inner retina, now known to be a characteristic of RP in humans and rodent animal models. Although extended survival times beyond 6 months might reveal such remodeling after IAA treatment, such long survival would not be practical for many studies. The second difficulty is the variability of the effect of the toxin, both between rabbits and occasionally even between the two eyes of a single rabbit. While the evidence thus far is that the toxin is selective for photoreceptors, the extent of the damage is not predictable.
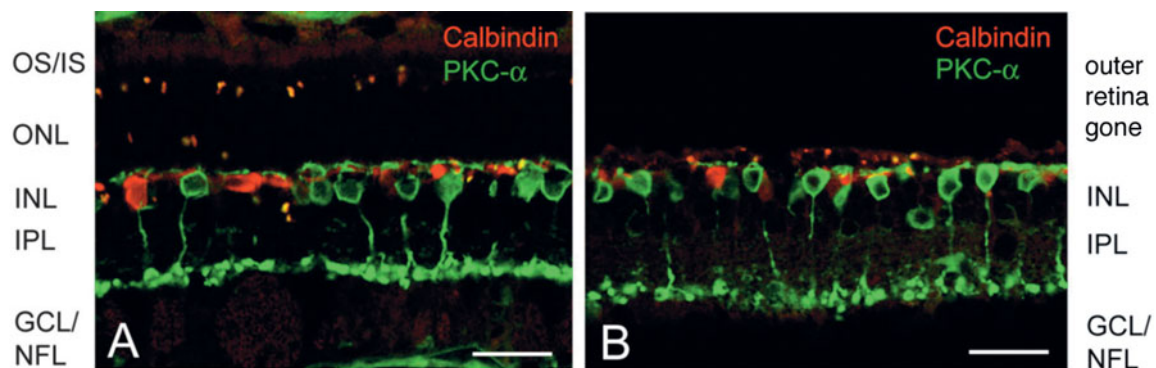

Fig. 6. Double labeling for calbindin D-28 (horizontal cells, in red) and protein kinase C-α (rod bipolar cells, in green) in retina #13, 30 days after IAA administration. Fig. 6A shows an undamaged region of this retina and Fig. 6B shows a damaged region of the same retina. In Fig. 6B, the outer retina is completely missing, and the horizontal cells and rod bipolar cell bodies are close to the retinal surface. Rod bipolar cell processes extend directly through the inner plexiform layer to arborize at its base in both damaged and undamaged retina. Scale bars = 25 microns. Abbreviations: OS/IS: inner and outer segments, ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL/NFL: ganglion cell layer/nerve fiber layer.
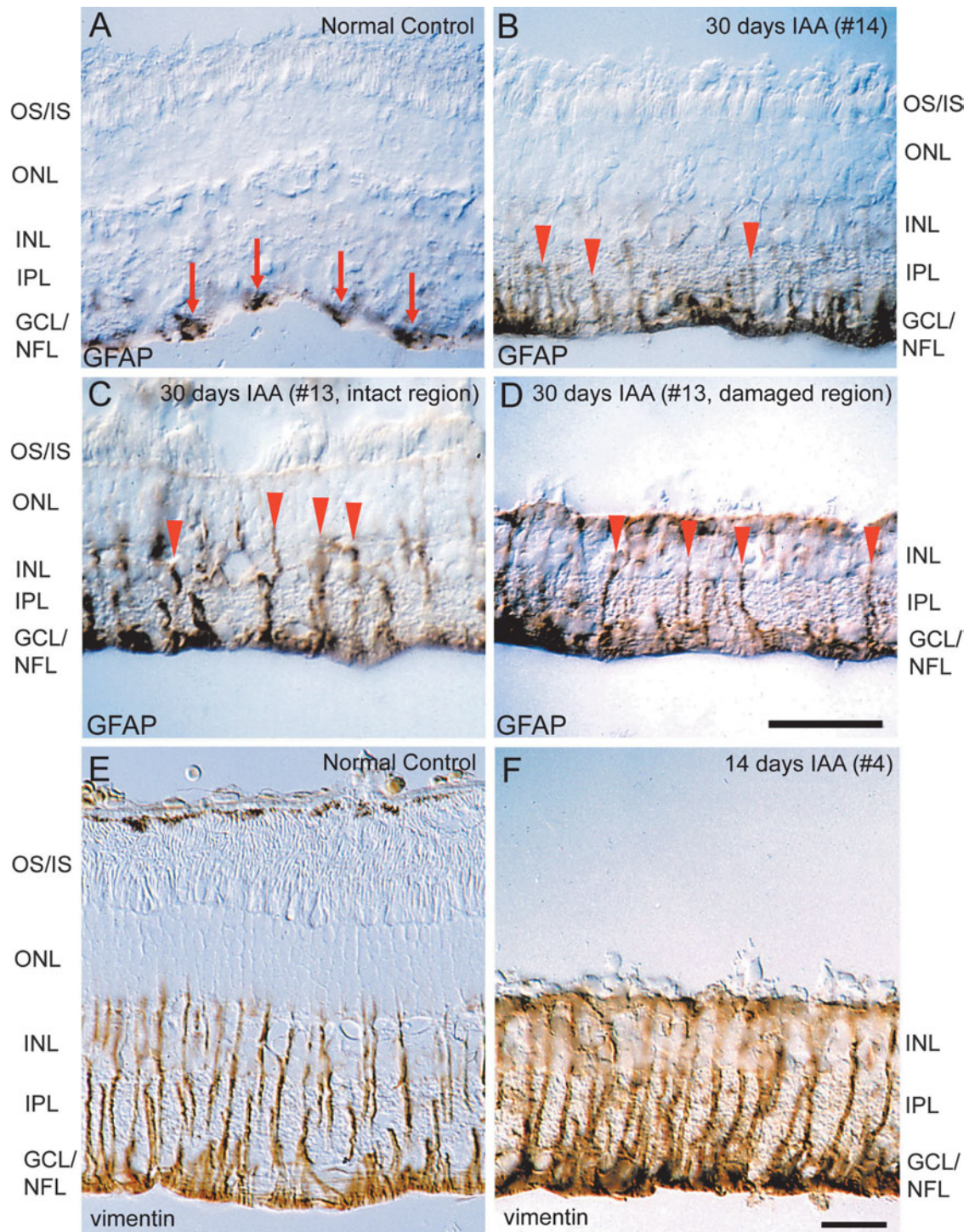
Fig. 7. The effect of IAA on glial cell immunoreactivity. Fig. 7A shows immunoreactivity for glial fibrillary acidic protein (GFAP) in a control retina (#1) and Fig. 7B shows GFAP immunoreactivity in a retina that was exposed to IAA 30 days before sacrifice (#14) but which did not have any quantitative signs of photoreceptor loss. In control retina limited GFAP labeling was found in the NFL (arrows), while in retina exposed to IAA the Müller cell stalks became mildly immunoreactive (arrowheads). Fig. 7C shows that GFAP labeling in Müller cell stalks is intense in the otherwise intact region of a retina that was damaged by IAA administered 30 days before sacrifice (#13), and Fig. 7D shows a similar pattern of labeling in the Müller cell stalks in the damaged region of the same retina. Fig. 7E shows that vimentin immunoreactivity labels Müller cell stalks and endfeet in control retina, and Fig. 7F shows vimentin labeling in the same structures in retina damaged by IAA (#14). In the absence of the ONL the vimentin-positive stalks and endfeet appear somewhat thickened, but the radial organization of the cells remains. Scale bar in D applies to panels A–D and equals 50 microns, scale bar in F applies to panels E and F and equals 25 microns. Abbreviations: OS/IS: inner and outer segments, ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, NFL: nerve fiber layer.

Increasing the dose of toxin is not practical because it increases overall mortality. However, the problem is somewhat offset by preliminary findings that electrophysiological measures such as the electroretinogram, are correlated with the anatomical effects of the toxin in a given rabbit (Franco et al., 2005), allowing one to pre-select affected eyes for further studies. Nonetheless, the lack of understanding of the source of the variability remains troubling.

Despite these limitations, we conclude that iodoacetic acid is a useful agent to produce selective, long lasting damage to the outer retina in the rabbit. It does not appear to provoke the robust glial activation that occurs in some other paradigms. The ventral retina is more vulnerable than other locations, which may be related to the pattern of the intrinsic retinal and choroidal vasculature in the rabbit. The abrupt photoreceptor damage caused by this toxin leaves specific cell populations intact in the inner retina, including rod bipolar cells and horizontal cells. As such, IAA provides a useful tool for eliminating photoreceptors, and IAA-treated animals may have uses for testing therapeutic interventions for retinal degenerations that rely on an intact inner retina.

Acknowledgments

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