Changes in the Expression of Specific Müller Cell Proteins during Long-Term Retinal Detachment

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Retinal detachments were produced in domestic cats by injecting fluid between the retinal pigment epithelium and neural retina. Retinas were allowed to remain detached for 30 or 60 days at which time the animals were killed. Tissue areas from detached and attached retinal regions from the same eye were processed for correlative biochemical and structural analysis, i.e. SDS-PAGE and Western blots of tissue homogenates were correlated with tissue processed for postembedding immunoelectron microscopy. Antibodies to six proteins were used as probes. Glial fibrillary acidic protein in Müller cells has previously been shown to increase after retinal detachment; here we show that vimentin, another intermediate filament protein present in Müller cells, also increases after detachment. In contrast, cellular retinaldehyde binding protein, cellular retinol binding protein, glutamine synthetase, and carbonic anhydrase C—all normally found in Müller cells—decrease after detachment. The results of this study indicate that retinal Müller cells dramatically alter their expression of proteins in response to retinal detachment.

Key words: Müller cells; retina; retinal detachment; glial fibrillary acidic protein; vimentin; cellular retinaldehyde binding protein; cellular retinol binding protein; carbonic anhydrase C; glutamine synthetase.

1. Introduction

A number of morphological changes occur in the neural retina when it is experimentally separated from the retinal pigment epithelium (RPE; Kroll and Maechemer, 1968; Anderson, Stern, Fisher, Erickson and Borgula, 1981; Erickson, Fisher, Anderson, Stern and Borgula, 1983). Photoreceptor outer segments rapidly degenerate to the point where only a few disorganized discs remain attached to the cilium; degeneration of cell processes, cell bodies and synaptic terminals occurs in outer nuclear (ONL) and outer plexiform layers (OPL; Erickson et al., 1983). After production of an experimental retinal detachment, there is also proliferation and migration of several cell types. Among the most reactive cells are the Müller cells, the specialized radial glia that extend the width of the retina from the outer to inner limiting membranes. By two days after detachment, Müller cell nuclei label with [3H]thymidine (Erickson et al., 1983) and can be seen migrating into the OPL and ONL (Erickson et al., 1983; Anderson, Guérin, Erickson, Stern and Fisher, 1986; Erickson, Fisher, Guérin, Anderson and Kaska, 1987). At this same time, Müller cells greatly elevate their level of RNA synthesis (Erickson, Fisher, Guérin, Anderson and Kaska, 1985) and show a build-up of intermediate filaments (Erickson et al., 1983, 1987). As the detachment time increases, the Müller cell processes expand within the OPL and ONL and sometimes extend into the subretinal space where they can interfere with photoreceptor outer segment regeneration after retinal reattachment (Erickson et al., 1983; Anderson et al., 1986a). Antibodies to specific Müller cell...
proteins were therefore used as probes to further delineate cellular and molecular changes that occur during a period of retinal degeneration induced by experimental detachment. We have previously reported the localization of these antigens in normal, unoperated cat retina (Lewis, Erickson, Kaska and Fisher, 1988). Because the detachment does not involve the entire retina, areas of attached retina from the eye with the detachment were compared to detached retinal areas.

The structural changes in the retina after detachment resemble those present in a variety of human and animal retinal disorders including: retinitis pigmentosa (Rayborn et al., 1985), macular disease (Eagle, 1984), diabetic retinopathy (Nork, Wallow, Sramek and Anderson, 1987), progressive rod-cone degeneration (Aguirre, Stramm and O'Brien, 1985), various retinal dystrophies in rats and mice (Dowling and Sidman, 1962; LaVail, 1981; Eisenfeld, Butt-Milam and Sarthy, 1984; Sarthy and Fu, 1988), responses to penetrating ocular injuries (Bignami and Dahl, 1979; Miller and Oberdorfer, 1981) and damage caused by constant light (Eisenfeld et al., 1984; Sarthy and Fu, 1988). Therefore, the changes described in this study are likely to be part of the retina's characteristic response to acquired or inherited degenerative diseases.

2. Materials and Methods

Cat retinas were experimentally detached from the retinal pigment epithelium for 30 \((N = 2)\) or 60 \((N = 2)\) days. The eyes were entrained to a 12:12 light cycle and were killed 4 hr after lights on, at which time several different areas from detached and attached retinas were processed for immunocytochemistry. SDS–PAGE and Western blot analysis. The procedure for producing the detachment has previously been published (Anderson et al., 1986a). Briefly, the lens was removed from the eye and the eye was allowed to heal for a minimum of 2 weeks at which time the vitreous was removed and a micropipet attached to an infusion pump was inserted between the neural retina and the RPE. The forces promoting retinal reattachment in the cat are strong enough such that in retinas with small tears, reattachment occurs spontaneously after about 7 days. In order to investigate the long-term detachments (without producing large retinal tears) we injected a 0:25% solution of Healon (sodium hyaluronate. Pharmacia) into the subretinal space through the micropipet: 30 and 60 days were chosen as possible end-points to some of the changes that occur with retinal detachment and to allow time for the formation of a subretinal scar.

Tissue for immunocytochemistry was cut into quadrants and fixed for 1 hr with 1% paraformaldehyde and 1% gluteraldehyde in sodium phosphate buffer. The retinas were then dehydrated in increasing concentrations of methanol and embedded in LR White resin (Polysciences). Uranyl acetate (2%) was added, en bloc, to 70% methanol during the dehydration as a secondary fixative to enhance contrast and to improve tissue preservation and immunolabeling of proteins (Erickson, Anderson and Fisher, 1987).

Rabbit anti-bovine-glial fibrillary acidic protein (GFAP, DAKO Corp.) and mouse anti-porine-vimentin (DAKO Corp.) were used at a dilution of 1:400. Rabbit anti-bovine-cellular retinol binding protein (CRBP) and cellular retinaldehyde binding protein (CRALBP: both gifts from John Saari) were used at a 1:200 and 1:400 dilution, respectively. Rabbit anti-chicken-glutamine synthetase (GS) and carbonic anhydrase C (CA-C: both gifts from Paul Linser) were used at a dilution of 1:600. All antibodies were diluted with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA: Sigma). Normal goat serum (NGS; Vector Labs; diluted 1:75 with PBS) was used as a blocking agent on each section before applying the primary antibodies. Goat anti-rabbit (GAR) or goat anti-mouse (GAM) IgG conjugated to 5 nm gold spheres (Janssen) was used at a 1:20 dilution for light microscopic immunocytochemistry (LM ICC). The 5 nm gold was amplified using a silver-enhancement technique (Janssen). Goat anti-rabbit IgG conjugated to 15 nm gold spheres was used at a dilution of 1:20 for single-label electron microscopic immunocytochemistry (EM ICC). For double-label experiments combinations of 5 nm and
15 nm or 15 nm and 30 nm gold spheres were used as secondary antibody tags, both at a 1:20 dilution.

Double-label immunocytochemistry with anti-GFAP and anti-vimentin has previously been described (Erickson and Fisher, 1988). Briefly the primary antibodies were combined and the mixture was added to the sections. Secondary antibodies were also combined and added to the sections. Since anti-GFAP was raised in a rabbit and anti-vimentin was produced using mice, GAR and GAM secondary antibodies, conjugated to differently sized gold spheres, were used to distinguish between the two primary antibodies.

Light microscopic immunocytochemistry was performed at room temperature using 1 μm thick sections taken from detached and attached retinas from the eye with a detachment. The sections, which had been placed on formvar-coated glass slides to prevent them from being washed off the glass surface, were then covered with NGS for 30 min, blotted, and incubated overnight with the primary antibody. The following day, sections were rinsed with PBS-BSA and the secondary antibody was added for 1 hr. Sections were subsequently rinsed with PBS, and the gold spheres were enlarged using the silver enhancing procedure. When the enhancing was complete, the sections were rinsed in PBS and distilled water and air dried. Sections of attached and detached retinas for each antibody were always processed together and enhanced for the same time period to ensure equal treatment.

Thin sections for EM ICC were cut from the same blocks of tissue that were used for LM ICC. The sections were first placed on nickel grids, incubated on drops of NGS for 30 min. blotted and incubated overnight on the primary antibody. The next day the grids were rinsed with PBS-BSA and transferred to drops of the secondary antibody for 1 hr. After rinsing with PBS-BSA and distilled water, the grids were stained with uranyl acetate for 15 min and lead citrate for 7 min, exposed to osmium tetroxide vapors (2% osmium in PBS) for 1 hr, and carbon coated in a vacuum evaporator.

Normal, detached and attached retinal tissue used for SDS–PAGE and Western analysis was homogenized in 0.02 M PBS containing 0.1 M phenylmethylsulfonylfluoride. 10 mM EDTA, 0.5% Triton X-100 and 2.55% SDS to inhibit protein degradation. Protein concentrations were determined by the BCA Protein Assay (Pierce Chemical Co.). A 50 μg sample of protein was analyzed by SDS-PAGE using a 7.5–20% gradient gel. Proteins of known molecular weights (Bio-Rad) were used as standards. Western blots were prepared by transferring the separated retinal proteins to nitrocellulose paper, blocking with 3% BSA in Tris buffered saline (TBS) and incubating overnight in primary antibody (diluted 1:100 in 1% BSA in TBS). The next day the blots were washed in Tween-Tris buffered saline (TTBS: 0.05% Tween 20) and incubated with horseradish peroxidase (HRP) labeled goat anti-rabbit or goat anti-mouse IgG (Bio-Rad) for 1 hr. Blots were again washed in TTBS and then TBS and placed in HRP Color Development Reagent (Bio-Rad) for 15 min. After distilled water rinse, the immunoblots were air dried. In order to allow comparisons of proteins from attached, detached and normal retinas, identical amounts of protein were run in adjacent gel lanes and transferred to a single piece of nitrocellulose for simultaneous immunoblotting.

3. Results

Immunocytochemical labeling of attached retinal regions with antibodies to GFAP [Figs 1(A), 2(A)] and vimentin [Fig. 2(B)] occurred predominantly over the Müller cell endfeet. Only occasionally did labeling extend beyond the inner plexiform layer. There was, however, a dramatic increase in the labeling of the Müller cells within the region of detachment. In both the 30- and 60-day detachments, anti-GFAP [Figs 1(B) and 2(C)] and anti-vimentin [Fig. 2(D)] labeling were present throughout the Müller cell cytoplasm, in some instances extending from the internal limiting membrane into the sub-retinal space between the photoreceptors and the RPE. Results from detachments were taken from the superior retina (which is thicker than the inferior retina) while the attached regions routinely came from inferior retina. This and the fact that detachment tends to produce some retinal thickening results in the retina appearing wider in sections from the area of detachment.
FIG. 1. Light microscopic silver enhanced immunocytochemistry using anti-GFAP on sections of attached (A) and detached retinas (B). In the attached retina, labeling is present predominantly over the Müller cell endfeet. After 60 days of detachment labeled Müller cell processes extend beyond the outer limiting membrane (arrowheads) into the subretinal space (SRS). The antibody labeling reaction product appears black against the red background of basic fuchsin counterstain. These figures can be used to provide orientation in the remaining figures showing results from sections that were not counterstained. (* vitreous cavity; INL, inner nuclear layer.) x 275.
Fig. 2. Light microscopic silver enhanced immunocytochemistry using anti-GFAP (A, C) and anti-vimentin (B, D) on sections of 60 day attached (A, B) and detached (C, D) retinas. The sections are serial to those in Fig. 1 but are unstained to allow the fine detail of the labeling pattern to be resolved. Anti-GFAP and anti-vimentin labeling is greatest over the Müller cell endfeet in attached retinas (A, B) but extends into the subretinal space (SRS) in detached retinas (C, D). (The RPE and choroid in (A) and (B) contains pigment granules and no labeling.) (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM.) × 275.
Using a double-label electron microscopic technique, labeling with anti-GFAP and anti-vimentin appeared over the clusters of intermediate (10 nm diameter) filaments in the Müller cells in the same pattern described above for the single-label procedures in both the detached and attached regions. Filaments were present in Müller cell cytoplasm within the ONL of detached retinas and labeled with anti-GFAP and vimentin [Fig. 3(B)]. No label was apparent in Müller cell cytoplasm within the ONL of attached retinas [Fig. 3(A)].
Fig. 4. Light (A, B) and electron (C, D) microscopic immunocytochemistry of anti-GS labeling in 60 day attached (A, C) and detached (B, D) retinas. In attached retinas labeling occurs predominantly in the Müller cells with the heaviest labeling over the endfeet (A, C). (The RPE and choroid in (A) contain pigment granules and no labeling.) After 60 days of detachment there is a dramatic decrease in the label present in the retina (B, D). (* vitreous cavity; INL, inner nuclear layer; arrowheads. OLM; SRS, subretinal space.) Light micrographs × 275; electron micrographs × 4100.
Fig. 5. For legend see opposite.

Fig. 6. For legend see opposite.
Immunolabeling with antibodies to GS, CAC, CRALBP and CRBP in attached retinal regions occurred throughout the Müller cell cytoplasm, with the heaviest labeling seen in the endfeet [Figs 4(A), 5(A), 6(A), 7(A)]. Anti-CAC and anti-CRBP labeling was also present in the photoreceptor nuclei and anti-CRALBP and CRBP labeling was found in the RPE cytoplasm. In the detached retinas, however, the labeling with all four antibodies dramatically decreased in the Müller cells [Figs 4(B), 5(B), 6(B), 7(B)]. Some anti-CAC labeling, however, was still apparent.

Fig. 5. Light microscopic immunocytochemistry of anti-CAC labeling in 60 day attached (A) and detached (B) retinas. In attached retinas labeling occurs in the Müller cells and photoreceptor nuclei. [The RPE and choroid in (A) contains pigment granules and no labeling.] After 60 days of detachment there is a decrease in CAC labeling in the retina although some label is still apparent in the outer nuclear layer. (* vitreous cavity: INL, inner nuclear layer: arrowheads, OLM, SRS, subretinal space.) x 275.

Fig. 6. Light microscopic immunocytochemistry of anti-CRALBP labeling in 60 day attached (A) and detached (B) retinas and the RPE in a region of retinal detachment (C). In attached retinas labeling occurs in the Müller cells and the RPE. [The RPE in (A) also contains pigment granules as does the unlabeled choroid.] After detachment there is a dramatic decrease in labeling in the retina. The RPE in regions where the retina is detached still labels although not as intensely as in the attached regions [as in (A)]. There are no pigment granules in the RPE in this region of the retina. (* vitreous cavity: INL, inner nuclear layer: arrowheads, OLM, SRS, subretinal space.) x 275.

Fig. 7. Light microscopic immunocytochemistry of anti-CRBP labeling in 60 day attached (A) and detached (B) retinas and the RPE in a region of retinal detachment (C). In attached retinas labeling occurs in the Müller cells, the ONL and the RPE. [The RPE in (A) also contains pigment granules.] After detachment there is a reduction in labeling in the retina although there is still some label present in the ONL. The RPE in regions where the retina is detached still labels but not as intensely as in the attached retinas. There are no pigment granules in the RPE in this region of the retina. (* vitreous cavity: INL, inner nuclear layer: arrowheads, OLM, SRS, subretinal space.) x 275.
Fig. 8. Control sections of light (A, B) and electron (C, D) microscopic immunochemistry from attached (A, C) and detached (B, D) retinas. There are pigment granules present in the RPE and choroid of the attached retina (A) that does not represent labeling. (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) Light micrographs × 275; electron micrographs × 4100.
MÜLLER CELLS AFTER RETINAL DETACHMENT

Western blots of homogenates from normal cat retinas showed single bands at the appropriate molecular weights for all the antibodies except that of anti-vimentin: anti-GFAP labeled a band at 51 kDa, anti-CRALBP at 33 kDa, anti-CRBP at 16 kDa, anti-GS at 45 kDa and anti-CAC at 31 kDa (Fig. 9). Anti-vimentin labeled one

in the photoreceptor nuclei; anti-CRALBP and CRBP labeling was still detected in the RPE although it was not as intense as in attached regions [Figs 6(C) and 7(C)]. The same results were obtained for all the antibodies using electron microscopic immunocytochemistry. For the sake of brevity, only the anti-GS labeling is shown [Fig. 4(C)] wherein the endfoot region of the Müller cells showed the heaviest antibody labeling in attached retina, and few if any gold spheres occurred over comparable areas in the region of detachment [Fig. 4(D)]. No label was present on control sections using either the light or electron microscopic techniques (Fig. 8). Similar results were obtained for both the 30- and 60-day time points.

Fig. 9. Coomassie Blue-stained SDS-PAGE gel of molecular weight standards (Lane A) and proteins of cat retinal homogenates (Lane B). Western Blots of normal cat retinal homogenates are in Lanes C–H. Immunoblotting reveals bands at the accepted molecular weights for each of the proteins: anti-GFAP labels a band at 51 kDa (Lane C), anti-vimentin at 57 and 49 kDa (Lane D) (see Discussion regarding the second band), anti-CRALBP at 33 kDa (Lane E), anti-CRBP at 16 kDa (Lane F), anti-GS at 45 kDa (Lane G), and anti-CAC at 31 kDa (Lane H).
Fig. 10. Coomassie Blue-stained SDS-PAGE gels of molecular weight standards are shown in Panel A and proteins of retinal homogenates from attached, detached and normal retinas are shown in Panel B. Lanes 1–3, respectively. Western Blots of anti-GS (Panel C: attached, detached and normal retinas are shown in Lanes 1–3, respectively) and anti-CAC (Panel D: attached, detached and normal retinas are shown in Lanes 1–3, respectively). Both GS and CAC show a reduction in the intensity of bands using detached retinas (Panels C and D, Lane 2) as compared to the attached and normal retinas.

band at the appropriate molecular weight (57 kDa) and another at approximately 49 kDa. Western blots of attached, detached and normal retinas using antibodies to GS, CAC (Fig. 10), CRALBP and CRBP (Fig. 11) showed a decrease of labeling intensity in the lanes containing samples of the detached retinas, while normal and attached retinas had labeled bands with similar density.

4. Discussion

Retinal Müller cells, a specialized form of astroglia, have been shown to become ‘reactive’ in a number of ocular diseases. For example, they are suspected of contributing to proliferative vitreoretinopathy by migrating into the vitreous cavity and proliferating (Laqua and Machemer, 1975; Clarkson, Green and Massof, 1977; Machemer, 1988). The cytoplasmic level of GFAP and intermediate filaments has been shown previously to increase in response to penetrating ocular injury (Bignami and Dahl, 1979; Miller and Oberdorfer, 1981), light damage (Eisenfeld, Bunt-Milam and Sarthy, 1984), inherited retinal degeneration (Eisenfeld et al., 1984), and retinal
MÜLLER CELLS AFTER RETINAL DETACHMENT

Fig. 11. Coomassie Blue-stained SDS-PAGE gels of molecular weight standards are shown in Panel A and proteins of cat retinal homogenates from attached, detached and normal retinas are shown in Panel B. Lanes 1–3, respectively. Western Blots of anti-CRALBP (Panel C; attached, detached and normal retinas are shown in Lanes 1–3, respectively) and anti-CRBP (Panel D; attached, detached and normal retinas are shown in Lanes 1–3, respectively). Both CRALBP and CRBP show a reduction in the intensity of bands using detached retinas (Panels C and D, Lane 2) as compared to the attached and normal retinas.

detachment (Erickson et al., 1987). In retinal detachment, there is an invasion of the interphotoreceptor space (subretinal space) by blood-borne cells and cells from the neural retina and RPE (Ohkuma, 1972; Inahara, 1973; Machemer and Laqua, 1975; Johnson and Foulds, 1977; Anderson et al., 1981; Anderson et al., 1986a; Erickson et al., 1983). Erickson et al. (1983) used morphological criteria to identify Müller cells as one participant in this response. The results of this study show that a population of GFAP- and vimentin-containing cells appears in the expanded subretinal space during a long-term detachment; labeled processes from these cells often can be traced across the width of the neural retina in a configuration unique to Müller cells. Since Müller cells are the only cells in the cat retina that contain both GFAP and vimentin (Erickson and Fisher, 1988) these results provide conclusive evidence that Müller cells are the retinal cell type whose processes invade the subretinal space after detachment.

Intermediate filaments

Vimentin, an intermediate filament protein (see Steinert and Parry, 1985, for review) has been shown by other investigators to be in the endfoot region of normal
Müller cells in species other than cat (Shaw and Weber, 1984; Schnitzer, 1985). As is the case with GFAP, the majority of vimentin is localized in regions which are invested with clusters of intermediate filaments. The data from the double-labeling experiments show that GFAP and vimentin appear to colocalize to the same clusters of filaments. Studies in glioma cells have shown that these two different intermediate filament proteins can also form copolymers in the same filament (Sharp, Osborn and Weber, 1982; Quinlan and Franke, 1983; Wang, Cairncross and Liem, 1984).

The vimentin antibody recognized a second band on Western blots at a lower molecular weight (49 kDa) in addition to the appropriate size for vimentin (57 kDa). It has been suggested that this represents proteolytic degradation of vimentin and not non-specific binding of the antibody (Ciesielski-Treska, Goetschy and Amis, 1984). The high degree of specificity of this antibody in the immunocytochemical experiments tends to strengthen this argument as does the fact that we routinely find only one band (at 57 kDa) on Western blots using rabbit retinal homogenates (unpubl. obs.).

The presence or absence of GFAP in normal Müller cells has been a controversial issue. While some investigations show it to be present under normal conditions (Bignami and Dahl, 1979; O'Dowd and Eng, 1979; Ohira, Oshima and Kikuchi, 1984; Björkland. Bignami and Dahl, 1985; Kivela, Tarkkanen and Virtanen, 1986; Erickson et al., 1987), others conclude that GFAP is expressed only in retinas experiencing some form of damage (Miller and Oberdorfer, 1981; Shaw and Weber, 1983; Eisenfeld et al., 1984; Okada, Matsumura, Yoshimura and Ogino, 1988) or in Müller cells grown in culture (Wakakura and Foulds, 1988). We have consistently found immunocytochemical evidence of GFAP in Müller cells of normal cat retinas, both in situ and in culture (Erickson et al., 1987; Lewis, Kaska, Vaughan and Fisher, 1988). The retinas we have classified as normal are from young adult cats (6 months to 2 yr of age) and show no identifiable signs of trauma or degeneration when examined by indirect ophthalmoscopy or light and electron microscopy. The differences reported in the literature may be due to the difficulty in visualizing the relatively small amount of GFAP found in Müller cell endfeet in the normal eye or reflect actual species differences in the expression of these proteins.

The dramatic increase in the expression of intermediate filament proteins represents an important structural response of the Müller cells to retinal injury. Intermediate filaments have been assigned putative functions in the differentiation and maintenance of cell shape, anchoring the nucleus to the cell membrane, and functional compartmentalization of the cytoplasm (Lazarides, 1980, 1987). All of these are relevant to Müller cell behavior after retinal detachment because they change their shape, their nuclei often translocate to new regions and they alter their expression of specific cytoplasmic proteins (Erickson et al., 1983, 1987). The specific function(s) of the intermediate filaments of Müller cells in normal and in pathological retinas, however, remains to be determined.

Müller cell enzymes

Glutamine synthetase and CAC are two enzymes that have been localized to the Müller cells in numerous species, including cat (Lewis et al., 1988), and are thought to play important roles in the physiology of these cells (see Moscona, 1983, and Ripps and Witkowsky, 1985 for reviews). When glutamate, a putative retinal neurotransmitter, is released from neurons it is actively sequestered by Müller cells where GS converts it to glutamine which can freely diffuse across cell membranes and be re-
used by neurons. Norenberg (1982) showed that, after producing lesions in the parietal lobes of rats, GS levels initially increased in astrocytes but eventually decreased to non-detectable levels. Decreased levels of GS activity have also been shown to occur in the retinas of dystrophic mice when compared to their normal littermates (LaVail and Reif-Lehrer, 1971). Although at that time it was not known which cells contained GS, it was concluded that GS was not confined to the photoreceptors and that there was possibly a decay of GS activity in the inner retinal cells. The reduction of GS in the region of detachment may reflect a reduction in synaptic activity and, therefore, enzyme substrate in the detached retina.

Carbonic anhydrase C affects Na\(^+\) and K\(^+\) movement and fluid balance (Carter, 1972). The enzyme converts CO\(_2\) and H\(_2\)O to H\(_2\)CO\(_3\) which spontaneously dissociates into HCO\(_3\)\(^-\) and H\(^+\); the proton serves as a regulator of intracellular pH. Disruption of carbonic anhydrase activity by acetazolamide results in altered Müller cell function and a decrease in the b-wave of the ERG in the elasmobranch retina (Hensley, Linser and Cohen, 1987). Decreases in the amount of CAC in the detached retina may have significant effects on the Müller cells' ability to regulate the extracellular environment. Interestingly, Kuwabara and Cogan (1960) showed an increase of oxidative enzymes in Müller cells within retinas that had a detachment. Maintaining normal levels of specific enzymes may therefore be important for proper function and even survival of retinal neurons during detachment and may play a role in the ability of the retina to recover functionally after reattachment. Down-regulation in the amount of these two enzymes in Müller cells may reflect a general decrease in their substrates in the detached retina.

**Retinoid binding proteins**

The retinoid binding proteins, CRALBP and CRBP have previously been localized by immunocytochemistry to both Müller cells and RPE (Bunt-Milam and Saari, 1983; Saari, Bunt-Milam, Bredberg and Garwin, 1984; Bok, 1985; Eisenfeld, Bunt-Milam and Saari, 1985; Anderson et al., 1986; Lewis et al., 1988). In addition to a decline in the expression of these proteins in Müller cells after detachment, the levels of CRALBP and CRBP in RPE also appear to be less when compared to adjacent RPE that is still attached to the retina. The correlation between the loss of outer segments and a decline in the levels of two vitamin A binding proteins in Müller cells in experimental retinal detachment is intriguing and this system may prove useful in helping to determine the currently unknown role of these proteins in the Müller cells.

Although antibodies to CRBP and CAC labeled predominantly a diffuse cytoplasmic component of the Müller cells, they also appeared to label the photoreceptor nuclei. CRBP has previously been found in the nucleus of RPE cells where it has been suggested it may be involved in the control of gene expression (Bok, Ong and Chytil, 1984; Chytil and Ong, 1984). Carbonic anhydrase C has also been found in the nuclear fraction of some cells (Karler and Woodbury, 1959). It was shown that nuclear staining may be fixation dependent; when unfixed tissue was used the staining was found only in the cytoplasm (Hanasson, 1967, 1968; Lutjen-Drecoll and Lonnerholm, 1981; Lutjen-Drecoll, Lonnerholm and Eichhorn, 1983). These results suggest that nuclear localization of CAC may be due to non-specific diffusion into the nucleus. Because the antibodies labeled a single band on the immunoblot, the nuclear staining most likely does not represent binding to some other molecular species although it is possible that these soluble proteins diffuse into the nucleus after a relatively mild fixation.
5. Conclusions

The immunocytochemical and biochemical analyses are consistent in showing that levels of intermediate filament proteins increase in the detached retina whereas a number of Müller cell enzymes and binding proteins decrease in response to retinal detachment. Significant differences between retina adjacent to the zone of detachment and normal retina in the opposite eye are not apparent. Therefore, these results, and all of our other results to date (Erickson et al., 1985; Erickson, Lewis, Anderson and Fisher, 1988), point toward the conclusion that detachment initiates a localized change in retinal metabolism that is not found in adjacent areas of attached retina.

The changes in protein expression described in these experiments occurred after a relatively lengthy detachment interval (30 to 60 days). Recent data, however, suggest that at least some changes (e.g. in GFAP and RNA synthesis) occur well before that time—as early as 3 days post-detachment (Erickson et al., 1985). We are currently examining the time of onset of these changes, the factors responsible for their initiation, and whether these changes once started, can be halted or reversed.

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MÜLLER CELLS AFTER RETINAL DETACHMENT


MÜLLER CELLS AFTER RETINAL DETACHMENT


