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Tritiated Uridine Labeling of the Retina: Changes After Retinal Detachment*

PAGE A. ERICKSON†, CHRISTOPHER J. GUÉRIN AND STEVEN K. FISHER

Neuroscience Research Institute, and the Department of Biological Sciences, University of California, Santa Barbara, CA 93106, U.S.A.

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As part of a study designed to examine the response of photoreceptor cells to outer segment injury (retinal detachment), the pattern of RNA labeling ([^{3}H]uridine incorporation) has been determined in detached cat retinas. Retinas were experimentally detached from the adjacent cellular layer (the retinal pigment epithelium: RPE) by injecting fluid into the extracellular space between the retina and RPE. Twenty-four hours before the animals were killed they received intravitreal injections of [^{3}H]uridine. Autoradiograms were prepared from plastic sections 1·0 μm thick taken from detached retinal regions and, because the detachments do not encompass all of the retina, from nearby attached retinal regions. Twenty-four hours after retinal detachment there is a decrease in labeling intensity of the photoreceptors and Müller's glia in the region of detachment (compared to cells in nearby attached regions). Seventy-two hours after retinal separation, the same result is obtained in the photoreceptors, but labeling intensity is greatly increased in both the nuclei and cytoplasm of Müller's glia. The decrease in [^{3}H]uridine labeling of the photoreceptors correlates with a decreased staining intensity of the cytoplasm and ultrastructural signs of necrosis. The striking change in the pattern and intensity of labeling of the Müller cells precedes extensive hypertrophy of these cells and the appearance within their cytoplasm of numerous 10-nm diameter filaments. Two weeks, and also 1 month, after detachment the pattern and labeling levels are similar to those observed 1 day after retinal separation. These data suggest a highly localized change in metabolism because the change in RNA labeling is restricted to the region of detached retina.

Key words: autoradiography; retina; retinal detachment; RNA; uridine.

1. Introduction

Photoreceptor cells degenerate after they are separated (detached) from the adjacent pigment epithelial cell layer (RPE; Kroll and Machemer, 1968; Machemer, 1968; Anderson et al., 1983; Erickson et al., 1983). If the two layers are reapposed, at least a portion of the photoreceptors (the outer segments) can undergo a certain degree of regeneration (Kroll and Machemer, 1969; Anderson et al., 1986). In detachments of more than a few days duration, photoreceptor cells begin to die and necrotic cells are found in the outer nuclear layer (Erickson et al., 1983). Besides these photoreceptor-specific changes, the Müller cells show multiple responses to detachment, including hypertrophy and growth into the subretinal space, proliferation, and changes in the expression of specific proteins (Anderson et al., 1983; Erickson et al., 1983, 1987; Lewis et al., 1989). Information about changes following detachment may provide us with a better understanding of the events leading to retinal degeneration and of conditions that either inhibit or promote regeneration in the retina.

Since one of the responses of neurons to injury is a change in RNA metabolism (Watson, 1965) we wanted to determine the pattern and levels of RNA labeling, as measured by [^{3}H]uridine incorporation, in experimentally detached retinas. By careful injection of solutions into the subretinal space, experimental detachments can be localized within a single quadrant of the eye (Frambach and Marmer, 1982; Anderson et al., 1983), thus allowing comparisons of attached and detached regions between (or within) quadrants. This type of analysis allows determining if changes extend beyond the region of detachment into adjacent areas that are still attached to the RPE. In this study, [^{3}H]uridine incorporation in attached and detached areas of retina were studied using tissue autoradiography. While there is a significant reduction in labeling of photoreceptors in the region of detachment, a large but transient increase in the labeling of Müller cells was identified at 3 days post detachment. A preliminary report of this data has been presented (Erickson et al., 1984).

2. Materials and Methods

Animals, Retinal Detachments and Labeling

Adult cats (6 months or older) were maintained on a lighting cycle (12 hr L:12 hr D) for at least 2 weeks prior to use in this experiment. Detailed methods of the lensectomy, vitrectomy and retinal detachment procedures have been published (Anderson et al., 1983, 1986). Briefly, the lens and vitreous were removed and a 0·5% solution of Healon (sodium hyaluronate; Pharmacia) was slowly injected (through a glass

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† For correspondence.

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micropipette) into the extracellular space between the photoreceptors and the RPE. The resulting retinal detachment radiated outward from the retinal hole produced by the micropipette. The retinas remained detached from the RPE for 1 day ($n = 1$), 3 days ($n = 2$), 2 weeks ($n = 1$) or 1 month ($n = 1$). Twenty-four hours before the animals were killed they received intravitreal injections of 200 μCi of [^{3}H]uridine (in 0·2 ml aqueous solution; 40–48 Ci mmol^{-1} = 1·48 – 1·78 TBq mmol^{-1} specific activity; Amersham). All animals were injected with [^{3}H]uridine 4 hr into the light period. Twenty-four hours after injection the animals were killed, the eyes enucleated, the anterior one-third of the globes excised and the eye-cups fixed by immersion in aldehydes. The eye-cups were subsequently divided into quadrants and processed for light-microscopic autoradiography. A detailed description of the methods are in a companion paper (Erickson and Fisher, 1990).

Autoradiography

Tissue sections 1 μm thick were cut using an LKB III ultramicrotome from areas of detached and nearby-attached retina. The sections were placed on glass microscope slides and dipped into a 1:1 solution of Kodak NTB-2 and distilled H_2O maintained at 43°C in the dark. The slides were exposed from 2 to 3 weeks at 4°C, developed for 2 min in D-19 (20°C), H_2O-washed, fixed, H_2O-rinsed and stained with azure II–methylene blue–toluidine blue in sodium borate. Slides from the detached and attached regions of each eye were processed together to assure equal treatment.

Grain Counts

Silver grains/rod photoreceptor nucleus were counted as in Erickson and Fisher (1990). All counts were performed using a Zeiss Universal light microscope at 2000 × magnification.

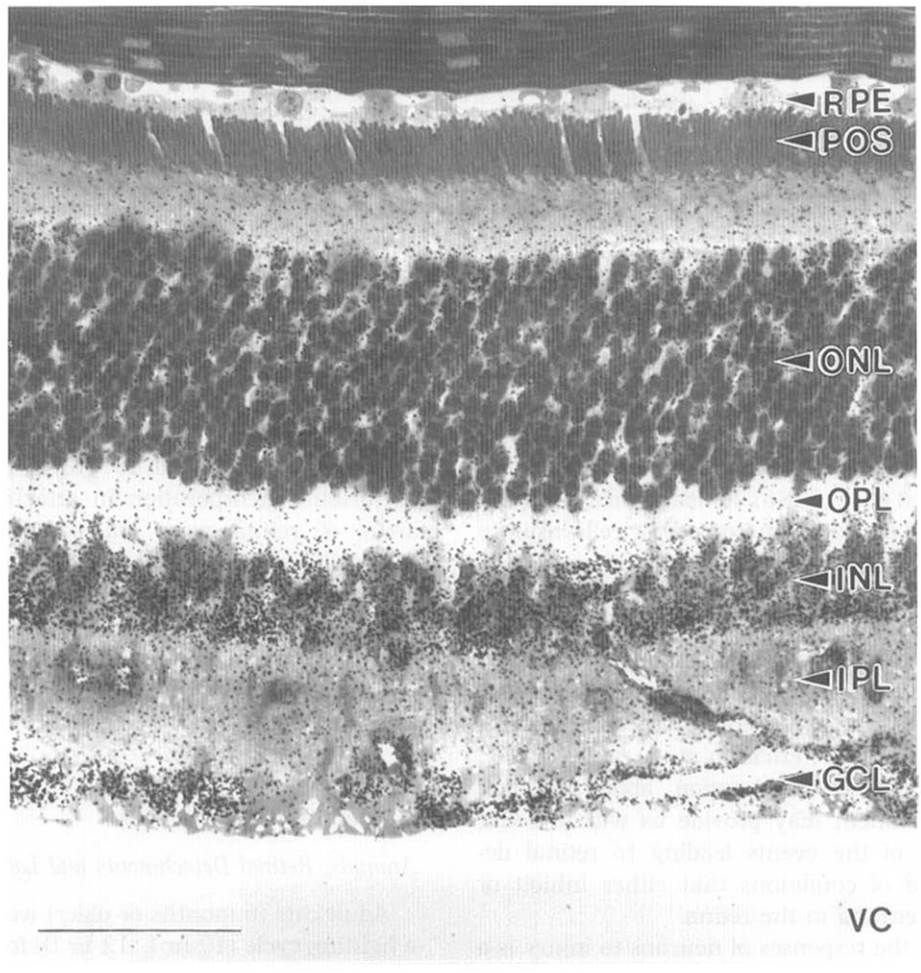

FIG. 1. This light-microscopic autoradiograph (bright field exposure) shows the pattern of retinal RNA labeling (uridine incorporation as indicated by the black grains) 24 h after injecting 200 μCi of [^{3}H]uridine into the vitreous cavity (VC). This autoradiograph is of an attached retinal region from an eye with a 1-day retinal detachment. The labeling pattern is identical to that observed in normal eyes. GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; POS = photoreceptor outer segments; RPE = retinal pigment epithelium. Bar = 50 μm.

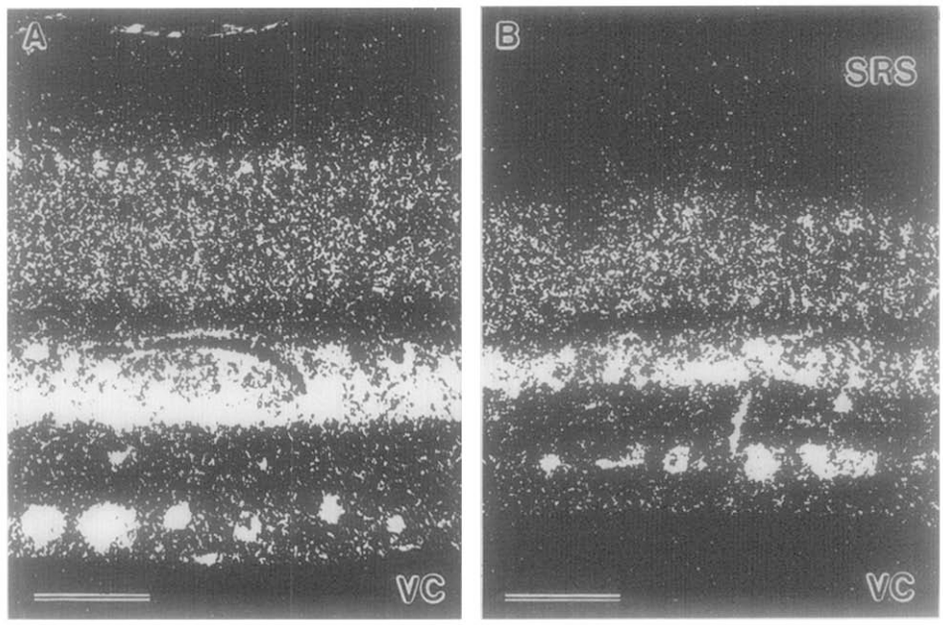

FIG. 2. These light-microscopic autoradiographs (dark-field exposures; [^{3}H]uridine) are from an eye with a 1-day retinal detachment. Silver-grain counts from these areas are presented in Fig. 3 (VC = vitreous cavity; bar = 50 μm). A, Attached retina near the area of detachment. This autoradiograph shows the pattern of RNA synthesis in a region where the retina remained attached to the RPE [1–2 mm from the detached area shown in Fig. 2(B)]. The pattern observed here is similar to that seen in the control retinas from normal eyes. B, Retina detached for 1 day. This autoradiograph is of retina that was separated from the RPE for 24 hr. Compare the labeling intensity seen in this detached retina with that observed in attached retina 1–2 mm away [Fig. 2(A)]. Vitreous cavity = VC; subretinal space = SRS.

3. Results

The labeling pattern in the attached retinal regions (Fig. 1) is identical to that observed in normal eyes (no retinal regions detached; Erickson and Fisher, 1990). By 1 day after retinal detachment, all cell types in the area of detachment show a decrease in uridine labeling (Fig. 2). There is approximately a 50% reduction in silver grains/rod nucleus in the detached region in comparison to rods in nearby-attached retina (Fig. 3, $P < 0{\cdot}001$ with Student's t-test).

Between 2 and 3 days after detachment, the pattern of uridine labeling changes. While there is still a lower level of labeling in the neurons, there is a dramatic increase in labeling seen in the Müller cells (Fig. 4). This increase in Müller cell labeling is located both in the nucleus and the cytoplasm. Müller cells, as well as neurons, in nearby attached retina retain their normal pattern and level of labeling.

Two weeks after detachment, the pattern and levels of labeling are similar to those observed at 1 day (compare Fig. 5 with Fig. 2). The Müller cells in the detached retina show labeling mainly over their nuclei and, like the neurons, they are labeled at a lower level than cells in nearby-attached retina (Fig. 5). Similar results were observed 1 month after detachment (data not shown).

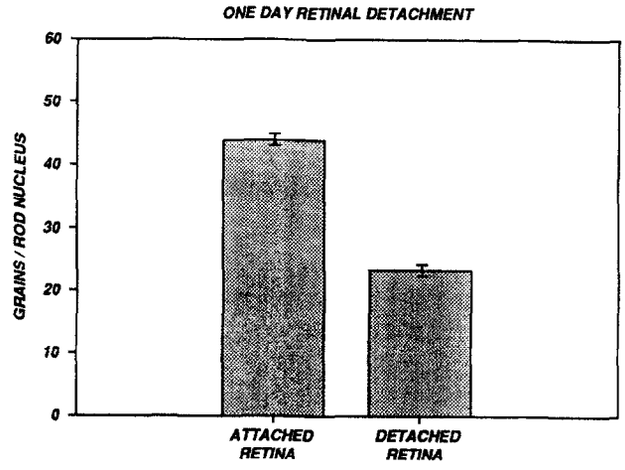

FIG. 3. This histogram shows the mean silver-grain counts (±S.E.M.) per rod photoreceptor nucleus from a cat with a 1-day detached retina. Grain counts were taken from the areas shown in Fig. 2 (attached retina and an area 1–2 mm away where the retina was detached). The labeling density in the detached retina is significantly reduced compared to the nearby attached retina ($P < 0{\cdot}001$; Student's t-test).

4. Discussion

Because of the regional variation in tritiated uridine labeling observed in the normal retina (Erickson and Fisher, 1990), data from the detached retina was always compared to nearby-attached retina within the same quadrant. The decrease in uridine labeling observed in retina detached for 1 day correlates with many structural indicators of change in retinal cells, including a decreased staining intensity of neuronal cytoplasm and ultrastructural changes usually associated with necrosis (most prominently in photoreceptor cells; Erickson et al., 1983). The increase in uridine labeling of Müller-cell nuclei and cytoplasm, in retina

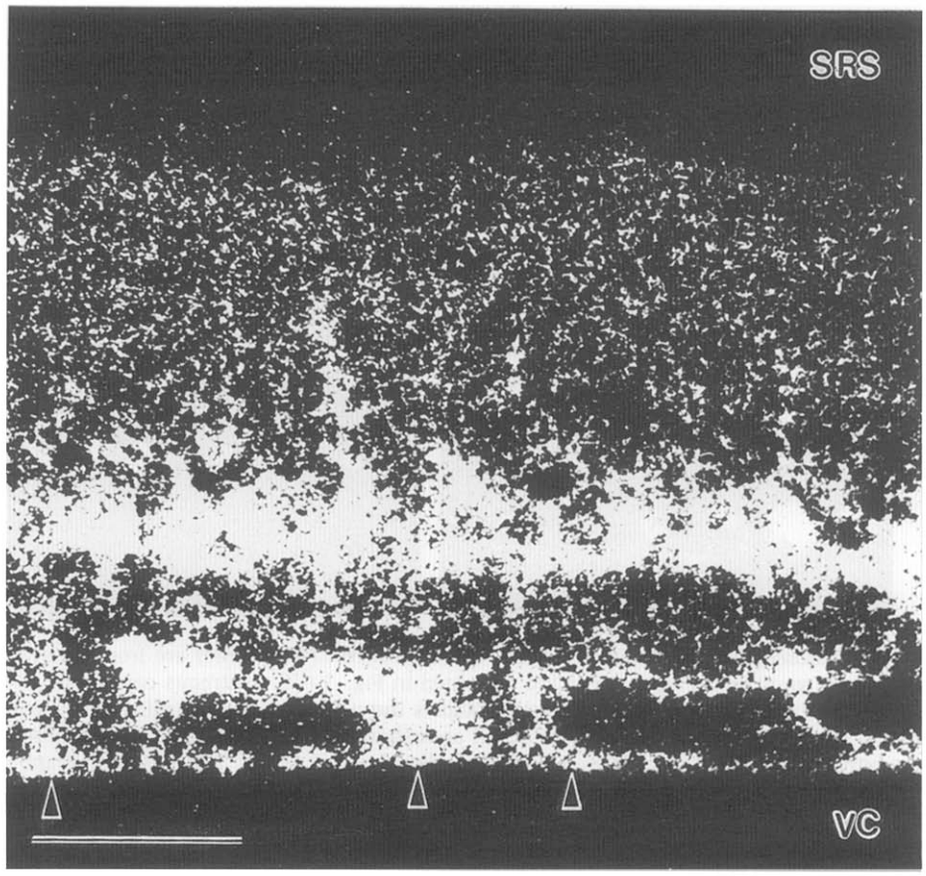

FIG. 4. This light-microscopic autoradiograph (dark-field exposure) of detached retina is from a cat eye with a 3-day retinal detachment; [^{3}H]uridine present for the last 24 hr. Note the change in the pattern and the intense labeling of the Müller cells (most easily seen in the Müller cell end-feet = arrowheads). Compare with retina detached for 1 day [Fig. 2(B)] and 2 weeks [Fig. 5(B)]. Vitreous cavity = VC; subretinal space = SRS. Bar = 50 μm.

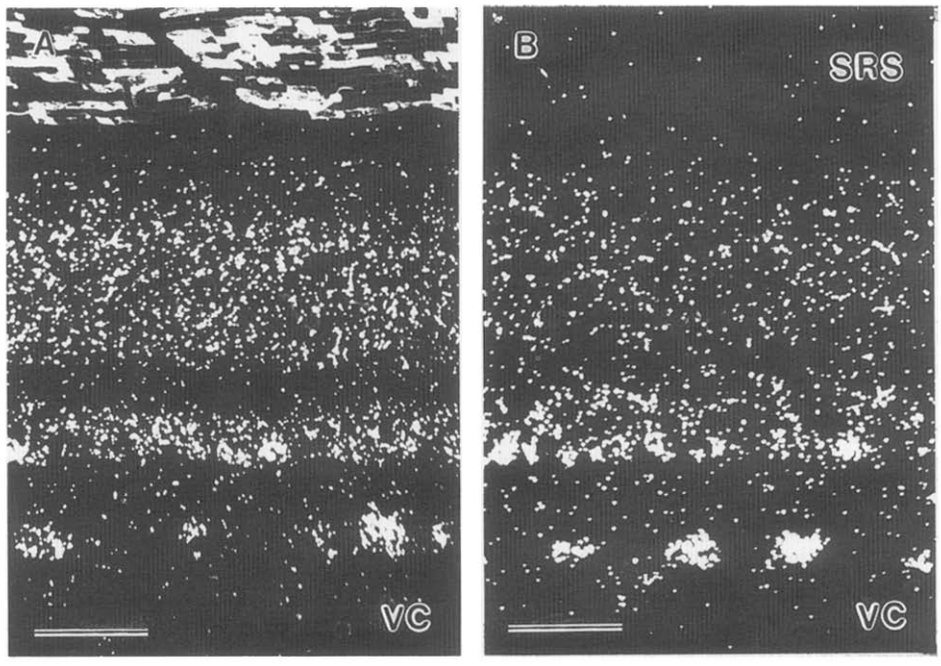

FIG. 5. These light-microscopic autoradiographs (dark-field exposures) are from a cat eye with a 2-week retinal detachment ([^{3}H]uridine present for the last 24 h; vitreous cavity = VC; bar = 50 μm). A, Attached retina near the area of detachment [2 mm from the detached area shown in Fig. 5(B)]. The pattern of RNA labeling in this region, where the retina remained attached to the RPE, is similar to that seen in normal eyes. B, Retina detached for 2 weeks. Compare the labeling pattern and intensity seen in this detached retina with that observed in the attached retina 2 mm away [Fig. 5(A)]. Also compare this labeling pattern with the similar pattern in 1-day detached retina [Fig. 2(B)] and the very different pattern seen in 3-day detached retina (Fig. 4). Subretinal space = SRS.

detached for 3 days, precedes extensive hypertrophy of these cells and correlates with an increase in the number of intermediate filaments made of glial fibrillary acidic protein (GFAP; Erickson et al., 1987) and vimentin (Lewis et al., 1989). Recent evidence indicates that the level of messenger RNA (mRNA) for GFAP increases, in mouse Müller cells, during retinal degeneration (Sarthy and Fu, 1988). Thus the increased levels of Müller-cell uridine labeling may reflect higher levels of newly synthesized mRNA for GFAP (and possibly other proteins such as vimentin). Indeed, our preliminary data show that there is an increase in GFAP mRNA in the Müller cells 3 days after detachment (Erickson et al., 1989).

Sometime between 3 days and 2 weeks after detachment the levels of uridine incorporation drop to about the levels observed at 1 day and this pattern persists in the 30-day detachment. Even though the GFAP and vimentin content of Müller cells in 1 month detachments remains elevated, the levels of other proteins are reduced (Lewis et al., 1989). Reduction in these proteins may be reflected in the overall reduction of RNA labeling. Virtually nothing is known about proteins synthesized by photoreceptors during an episode of detachment. The decreased RNA labeling may be due to decreased RNA synthesis or increased RNA turnover. Either, or both, of these possibilities would lead to the results observed in this study.

A highly localized change in metabolism is indicated by these results because the change in RNA labeling is restricted to the region of detached retina. Similar localization has been observed for morphological changes, cell death, and changes in protein expression (Erickson et al., 1983, 1987; Lewis et al., 1989). It has been shown that rod outer segment debris can inhibit RNA synthesis in lymphocyte cultures, and that this inhibition can be counteracted by vitamin E and other antioxidants (Gery, 1980). Because the rod outer segments are disrupted only in the region of detachment, they may play a role in the inhibition of RNA synthesis observed in detached retina. If this occurs, antioxidants may help to protect retinal cells during detachment. There also may be a general reduction in retinal metabolism in the region of detachment due to the separation from the RPE and the adjacent choroidal circulation (see, for example) Steinberg, 1987). This is indicated by the correlation between the degree of separation of the retina from the RPE and the degree of degeneration within the retina (Machemer, 1968; Erickson et al., 1983).

The increased RNA labeling of the Müller cells at 3 days may be due to increased RNA synthesis or decreased RNA turnover (or both). If stimulation of RNA synthesis is occurring in the Müller cells, it may be the result of a release of some trophic factor(s) or even a release of inhibition (Nieto-Sampedro, 1988) within the region of detachment. The results obtained here help us to further define changes occurring in the retina as a result of detachment. Understanding the role of these changes in this induced retinal degeneration may help us to understand this and other visual degenerative diseases.

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