

Photoreceptor Degeneration in Vitamin A Deprivation and Retinitis Pigmentosa: the Equivalent Light Hypothesis

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Long-term exposure of the retina to constant illumination is known to produce irreversible degeneration of photoreceptors. We propose that similar mechanisms may be involved in photoreceptor degeneration produced by vitamin A deprivation and some forms of retinitis pigmentosa (RP). Evidence is reviewed suggesting that the free opsin present during vitamin A deprivation or the mutated opsin present in some forms of RP excite the visual transduction cascade. This would produce a constant 'equivalent light' that triggers photoreceptor degeneration. Continuous real or equivalent light may produce outer segment degeneration by interfering with circadian processes, such as protein synthesis and disc shedding and lead to the loss of photoreceptors including those not expressing the mutant gene.

Key words: photoreceptors; degeneration; vitamin-A deprivation; light damage; retinitis pigmentosa; adaptation; circadian rhythm.

Introduction

One of the most common causes of blindness is the selective degeneration of photoreceptors. This occurs in a variety of disorders including vitamin A deprivation and the group of diseases known as retinitis pigmentosa (RP) (Tso, 1989). Vitamin A deprivation is widespread in developing countries (Duke-Elder and Dobree, 1967) and can be caused by dietary deficiency or by digestive malabsorption (Perlman et al., 1983; Kemp et al., 1988). The lack of vitamin A produces an initial decrease in rod sensitivity (night blindness) and leads eventually to a complete loss of both rod and cone outer segments (Dowling and Gibbons, 1960; Hayes, 1974; Kemp et al., 1989). RP is an inherited disorder that has several forms: autosomal dominant, recessive or X-linked (Heckenlively, 1988). It has been shown that some forms of the disease are due to mutations in the rod outer segment proteins rhodopsin and peripherin, but the genetic defect of other forms has not yet been determined (see Humphries, Kenna and Farrar, 1992). Although the genetic origins of RP are diverse, most forms of RP produce a similar sequence of abnormalities. Initially there is a decrease in rod sensitivity. This is followed by a loss of rod outer segments and night blindness, much like that produced by vitamin A deprivation. With time, cone function is also lost and complete blindness occurs by the age of 50–80.

Despite considerable progress, the mechanism of photoreceptor degeneration in these diseases remains unclear. We believe that an important clue is the observation that photoreceptor degeneration similar to that which occurs in vitamin A deprivation and RP can be induced by long-term, continuous exposure to

light (Noell et al., 1966; Noell and Albrecht, 1971). This raises the possibility that defects in rod function generate a continuous signal equivalent to that produced by light, which sets in motion the same degenerative processes produced by light itself. In this paper we analyse this 'equivalent light' hypothesis. We also explore a related hypothesis that continuous real or equivalent light leads to degeneration by interfering with circadian processes, such as the shedding of rod discs. The long-term inhibition of these processes by equivalent light could lead to the degeneration of photoreceptors, including even those that do not express the mutant gene.

Constant Light Produces Degeneration

There is considerable evidence that light can produce degeneration of the photoreceptors (Noell, 1980). There are several forms of light damage, which probably work by different mechanisms (Noell, 1980; Williams and Baker, 1980). Some forms of light damage are produced by brief exposure to bright light and may involve direct damage by heating or oxidation. Other forms can be produced by dim lights, such as room light, if given uninterruptedly for several weeks (LaVail, 1980; Rapp and Williams, 1980). It is the latter form of light damage which we think may be relevant to understanding the degeneration caused by vitamin A deprivation and RP.

Many of the first reports of light-induced photoreceptor degeneration showed that albino rats were particularly sensitive to damage by constant light (Noell et al., 1966). It has subsequently been shown, however, that pigmented rats are less sensitive to

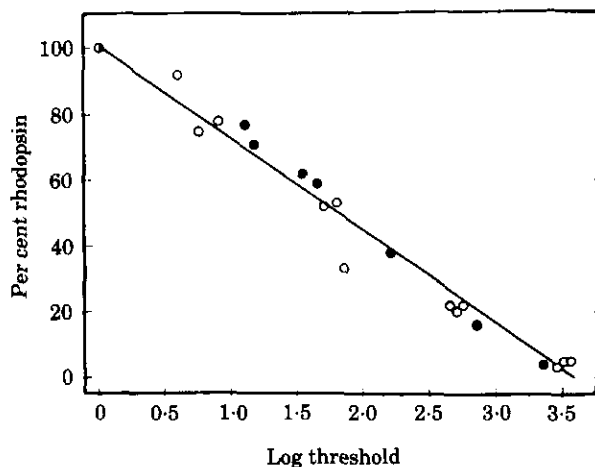


FIG. 1. The percent rhodopsin concentration of the retina of the rat eye plotted as a function of the \log_{10} of the visual threshold. Threshold (inverse of sensitivity) was defined as the intensity of light required to give a just detectable erg. The erg was measured from intact animals with surface electrodes, and rhodopsin concentration was determined spectrophotometrically after extraction of the pigment from isolated retinas. Measurements were made either from dark-adapted animals maintained from birth on a vitamin-A-deficient diet supplemented with vitamin A acid (●), or from animals maintained on a normal diet but subjected to previous bright bleaching illumination (○). The line through the data points is given by the equation $\log_{10} (I_1/I_0) = 3.6 (R_0 - R_1)/R_0$, where I_0 and R_0 are the thresholds and rhodopsin concentrations in dark-adapted animals raised on normal diets, and I_1 and R_1 are the thresholds and rhodopsin concentrations from vitamin-A-deprived or bleached animals. Taken with permission from Dowling and Wald (1960).

damage only because ocular pigmentation prevents light from reaching the retina. If care is taken to ensure comparable rhodopsin bleaching, albino and pigmented animals are equally affected by constant light (Rapp and Williams, 1980). Susceptibility to constant light has been demonstrated in a wide variety of animals and appears to be a universal feature of both vertebrate and invertebrate (Zinkl et al., 1990) photoreceptors. The action spectrum of the light damage has the approximate form of the rhodopsin absorption spectrum (Noell et al., 1966; Williams and Howell, 1983), suggesting that light damage is initiated by rhodopsin. The resulting activation of the phototransduction cascade must somehow set in motion degenerative processes leading to the death of both rods and cones.

Vitamin A Deprivation

Vitamin A deprivation may also lead to continuous activation of the phototransduction cascade. Rhodopsin is composed of a vitamin A derivative, 11-*cis* retinal, linked to a protein moiety, opsin. During vitamin A deprivation, there is a decrease in the concentration of rhodopsin in the outer segment and an increase in the concentration of opsin unbound to

chromophore (Dowling and Wald, 1958; Engbretson and Witkovsky, 1978). It has long been thought that opsin might be capable of stimulating the visual cascade (Barlow, 1964; Rushton, 1965). Recent experiments have demonstrated this directly. Opsin generated by bleaching is able to activate the guanylyl phosphodiesterase (Cornwall and Fain, 1992), although with an efficiency 10^{-7} – 10^{-6} less than the active form of rhodopsin (R^*) (see Fain and Cornwall, 1993). Despite this inefficiency, the excitation produced by opsin can still be substantial if enough opsin is present. For instance, if only 10% of the 10^9 – 10^{10} pigment molecules in a frog rod are present as opsin, their combined effect would be equivalent to a steady light of 10–100 R^* (assuming a mean lifetime of R^* of 1 sec). Since rods respond to even a single R^* (Baylor, Lamb and Yau, 1979), an opsin signal equivalent to 10–100 R^* s sec^{-1} would be substantial.

During vitamin A deprivation, the fraction of the pigment in the opsin state can be much greater than 10% (Dowling and Wald, 1958; Engbretson and Witkovsky, 1978). Since we know from bleaching experiments that opsin generates an equivalent light, it would seem likely that an equivalent light is also present during vitamin A deprivation. This argument is supported by erg recordings from vitamin-A-deprived animals (Dowling and Wald, 1960). If the changes in sensitivity at various stages of vitamin A deprivation are compared to the percent decrease in rhodopsin concentration (and therefore percent increase in the concentration of opsin), the resulting curve (Fig. 1) closely corresponds to that generated by bleaching various amounts of rhodopsin. This comparison provides evidence that the opsin generated by vitamin A deprivation excites and adapts like the opsin generated by bleaching.

Figure 1 illustrates a property of desensitization during bleaching and vitamin A deprivation that will also be relevant when we consider changes in sensitivity during RP. This figure shows the relationship between rhodopsin concentration and threshold ($1/\text{sensitivity}$) and indicates that the reduction in sensitivity is far greater than expected from the reduction in photon absorption (i.e. the reduction in quantum catch). Recent studies of bleaching adaptation (Cornwall and Fain, 1992) indicate that this extra adaptation produced by opsin is due to a modulation of the biochemistry of transduction similar to the one that is produced by real light (see Fain and Matthews, 1990).

Retinitis Pigmentosa

Evidence that an equivalent light is generated in one form of RP comes from the recent demonstration by Robinson and co-workers that mutation of the opsin lys²⁹⁶ produces a protein which continuously activates the transduction cascade even in the dark (Robinson

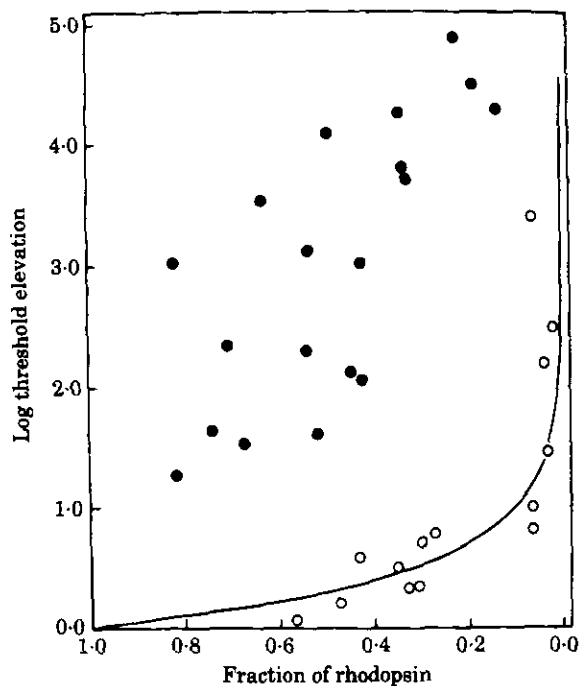


FIG. 2. Log_{10} elevation of visual threshold as a function of the fraction of rhodopsin concentration from 11 RP patients. The visual threshold was measured psychophysically and is defined as the intensity of a 525 nm light just bright enough to be detected. The fractional rhodopsin concentration was measured *in situ* by reflection densitometry. Open circles (O) are from three patients for whom the increase in threshold could be accounted for by the decrease in the fraction of rhodopsin. Curve gives relationship for increase in threshold predicted solely from the decrease in quantum catch. Other data points (●) are from eight patients for whom the increase in threshold was much larger than predicted from the decrease in rhodopsin concentration. Several data points are shown for each patient since measurements of threshold and rhodopsin concentration were made at several different retinal locations, and these were plotted separately. Taken with permission from Perlman and Auerbach (1981).

et al., 1992). This mutation is known to cause a rare form of autosomal dominant RP (Keen et al., 1991).

Continuous activation of the transduction cascade may also occur in other forms of RP. Perlman and Auerbach (1981) found that a significant number of RP patients show a reduction in sensitivity much larger than can be accounted for by the loss in quantum catch (see Fig 2, ●). Similar findings have been reported by Ernst and co-workers (1986) and by Kemp, Jacobson and Faulkner (1988). These measurements were made at an early stage in the progress of the disease, at which the rhodopsin concentration was reduced but still measurable, i.e. the photoreceptors had not completely degenerated. As discussed above, sensitivity loss greater than predicted by the reduction in quantum catch would be expected if transduction were being continuously activated by an 'equivalent' light.

Another indication that spontaneous activation of transduction occurs in many forms of RP comes from the reports of the patients themselves (see for example

Heckenlively, 1988; Barlow, 1990). Many patients complain of seeing spontaneous light flashes or constant bright light in the absence of any actual visual stimulus. In addition, Barlow (1990) measured the pupil size in a group of dark-adapted RP patients and found it to be highly constricted, with almost no overlap with normal dark-adapted controls. These results suggest that the retinas of RP patients may be signalling the presence of 'equivalent light' to the pretectal nuclei responsible for pupil constriction.

RP exists in many forms, and there is reason to suspect that not all are produced by continuous activation of transduction. Degeneration of the photoreceptors can also occur as a result of improper assembly of the outer segments (Fliesler, Rapp and Hollyfield, 1984). Some of the rhodopsin mutations that cause RP may form a non-functional protein that is not transported to the plasma membrane (Sung et al., 1991; but see Olsson et al., 1992). Other forms of RP result from a molecular defect in the structural protein peripherin (Farrar et al., 1991; Kajiwarra et al., 1991) leading to progressively smaller outer segments. In these cases where structural problems are involved, the decrease in visual sensitivity would be expected to be linearly proportional to the loss in outer segment rhodopsin, and there are some forms of RP where such a relationship has been directly demonstrated (Ripps, Brin and Weale, 1978; Perlman and Auerbach, 1981; Ernst et al., 1986; Kemp et al., 1988).

In such cases, RP is probably not due to continuous activation of the transduction cascade; however, an equivalent light signal produced in a different way may still trigger degeneration. To understand why this might be so, recall that rods are kept depolarized in the dark by a constant influx of cations into the outer segment (Yau and Baylor, 1989). Light reduces this dark current, and the resulting hyperpolarization of the receptor membrane turns off the release of neurotransmitter, thereby signaling to second-order neurons that light is present (see Dowling, 1987). A similar equivalent light will be generated by a photoreceptor whose outer segment has degenerated, because there will be no dark current and the inner segment will be hyperpolarized, (Bader, MacLeish and Schwartz 1978; Bader, Bertrand and Schwartz, 1982). This signal could trigger degeneration of other photoreceptors, including those that do not express the mutant gene (see below).

If desensitization in RP patients were caused by activation of transduction like that occurring in background light, the electrical activity recorded from a dark-adapted eye of an affected individual might be expected to resemble that recorded from a light-adapted eye of a normal individual. The dark-adapted photoreceptor responses should have a shorter time-to-peak in affected eyes than in the eyes of normal patients (Fain and Cornwall, 1993). We have been unable to find reports of such changes in time-to-peak, and most measurements of electrical activity from RP

patients show just the opposite: the b-wave of the electroretinogram (erg) has slower than normal kinetics (see Berson, 1987; Heckenlively, 1988). Since the erg is recorded in most cases long after degeneration has begun, it is possible that degeneration produces a slowing in the photoresponse kinetics, separate from that normally produced by light adaptation. Furthermore, the b-wave reflects the kinetics of the responses of second and third-order neurons in the retina in addition to photoreceptors, and it is possible that the slow kinetics of the b-wave are due to the effect of the disease on synaptic transmission in the retina (Ripps, 1982).

Equivalent Light and Circadian Rhythms

There are many possible mechanisms by which an equivalent light might trigger the degeneration of photoreceptors. However, one possibility, the inhibition of circadian processes, seems particularly plausible. A widespread (and perhaps universal) feature of circadian processes in vertebrates is that they can be inhibited by constant light (Pittendrigh, 1981), and it is possible that they may also be inhibited by a constant equivalent light. Many cellular processes in the retina are under the control of a circadian oscillator, located in the eye itself (Hollyfield and Basinger, 1978; Cahill, Grace and Besharse, 1991; Cahill and Besharse, 1992). These circadian processes include synthetic events, such as (in mammals) the synthesis of opsin (Matsumoto and Bok, 1984; Korenbrot and Fernald, 1989), and degradative events, such as the phagocytosis of rod disc membranes by the retinal pigment epithelium (LaVail, 1976). It has been directly shown that constant light inhibits rod disc phagocytosis (Goldman, Teirstein and O'Brien, 1980). Moreover inhibition of phagocytosis is sufficient to cause retinal degeneration, as demonstrated in the RCS mouse (Mullen and LaVail, 1976). It thus seems plausible that a constant equivalent light could cause retinal degeneration.

A puzzling aspect of RP is that a molecular defect in a rod protein can trigger the degeneration of cones (see Humphries et al., 1992). This becomes understandable if degeneration involves the inhibition of circadian processes. The retinal pathways that control these processes (reviewed in Besharse, Iuvone and Pearce, 1988) involve amacrine cells which release dopamine and thereby affect circadian processes in both rods and cones. This release of dopamine is itself controlled by light. In *Xenopus* retina, there is evidence that a circadian process in cones can be affected by signals coming from rods (Besharse and Witkovsky, 1992). The literature thus points to a pathway by which the generation of an equivalent light in rods could affect circadian processes in cones. Recent experiments using mosaic retinas are consistent with involvement of a complex pathway in retinal degeneration. These

experiments show that patches of normal photoreceptors degenerate because of the presence of nearby patches of photoreceptors containing mutant rhodopsin (Huang et al., 1993).

Further Experimental Tests

The equivalent light hypothesis makes many testable predictions. If vitamin-A-deprived animals are desensitized because of an equivalent light produced by opsin, the dark-adapted rods from these animals should behave like bleached rods, i.e. have a reduced dark current, lower sensitivity, and higher rates of dark PDE and cyclase activities (Fain and Cornwall, 1993). The equivalent light hypothesis makes similar predictions for the forms of RP where constitutive activation of the cascade is suspected. The best way of testing this possibility would be through direct recording from photoreceptors of transgenic animals.

As much as 20–30% of autosomal dominant RP can be attributed to defects in rhodopsin (Dryja et al., 1991). The mutation can occur at a multiplicity of amino acid residues (over 30 have been identified) (Humphries et al., 1992), and it remains unclear why rhodopsin should be sensitive to changes at so many different sites. The rhodopsin molecule is normally remarkably 'quiet' in darkness—the halftime for spontaneous activation of a rhodopsin molecule in a primate rod is approximately 420 yr (Baylor, Nunn and Schnapf, 1984). This is probably the result of strong evolutionary pressure. We suggest that many rhodopsin mutations may lead to increased spontaneous activation of the transduction cascade, as has already been demonstrated for the lys²⁹⁶ mutation (Robinson et al., 1992). Other mutations in rhodopsin might result in an increased activation of transduction through various mechanisms. These include; (1) increased spontaneous isomerization; (2) decreased efficiency of inactivation of rhodopsin after light excitation; (3) an increased concentration of opsin as the result of a change in the equilibrium between opsin and 11-*cis* retinal; and (4) changes in rhodopsin that affect its interaction with transducin. In addition, changes in proteins that interact with rhodopsin (like transducin or rhodopsin kinase), mutations in structural proteins (like peripherin), or alterations in the pathway that supply retinal (e.g., retinal binding proteins) could lead to continuous activation of transduction. The resulting constitutive activity could be quite small and still have a dramatic effect on the rod, as we have previously argued. New, more sensitive *in vitro* assays for constitutive rhodopsin activity may have to be developed to test this notion.

The equivalent light hypothesis for RP suggests several therapeutic strategies. In cases where rod transduction is constitutively active, drugs which interfere with rod transduction might be therapeutic. If inhibition of circadian processes is involved, as recent results are beginning to suggest (Agarwal et al.,

1992), it might be possible to block the effect of equivalent light on the circadian process.

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