

# Light, $\text{Ca}^{2+}$ , and Photoreceptor Death: New Evidence for the Equivalent-Light Hypothesis from Arrestin Knockout Mice

Gordon L. Fain<sup>1,2</sup> and John E. Lisman<sup>3</sup>

In this issue of Investigative Ophthalmology and Visual Science, Chen et al.<sup>1</sup> show that light exposure can greatly accelerate light damage in arrestin knockout mice. Arrestin and its splice variant p44 are soluble proteins that bind to phosphorylated rhodopsin and prevent the binding of rhodopsin to the G-protein transducin. Arrestin therefore plays a key role in turning off the visual cascade, and animals without arrestin show greatly prolonged light responses.<sup>2</sup>

Chen et al.<sup>1</sup> show that when arrestin knockout mice are placed in constant light too dim to produce degeneration in normal animals, the photoreceptors rapidly degenerate; but if animals are kept in darkness, no degeneration occurs. These results provide strong evidence that light itself, in conjunction with the slow turnoff of the photoreponse in the arrestin knockout animals,<sup>2</sup> is directly responsible for the degeneration of the photoreceptors. Arrestin knockout mice also show some evidence of degeneration in cyclic (12-hour light-12-hour dark) light, but the loss of photoreceptors is very slow. This slow rate of degeneration is broadly consistent with the progression of Oguchi disease in humans, a form of stationary night blindness that in some patients is apparently produced by mutations in the arrestin gene.<sup>3</sup>

The results of Chen et al.<sup>1</sup> raise the important question of how light exposure produces photoreceptor death. Although it has long been known that continuous exposure to light can produce degeneration, the significance of this observation has been unclear, first because degeneration is much more pronounced in albino animals than in pigmented animals,<sup>4</sup> and second because there is no agreement about how light damage occurs. In many previous reports, the intensity of the continuous light used to produce degeneration was high enough to produce a nonspecific, toxic effect of illumination. The experiments of Chen et al.<sup>1</sup> show that in arrestin knockout animals, light can produce degeneration even in pigmented animals at intensities that have no effect on normal pigmented mice. It is therefore very unlikely in these experiments that light had some nonspecific effect such as photodynamic damage. A more probable explanation is that in normal pigmented animals, the light was not bright enough to saturate the photoreponse, but in arrestin knockout animals, the pronounced decay of the single-photon response<sup>2</sup>

produced a more strongly maintained suppression of the photocurrent. As Chen et al.<sup>1</sup> concluded, the degeneration in arrestin knockout animals is probably caused by constitutive activation of the visual cascade.

We have previously proposed<sup>5,6</sup> that degeneration in continuous light and in certain forms of retinitis pigmentosa may be caused by constitutive activation of the photoreceptor. This equivalent-light hypothesis of retinal degeneration has received recent support, not only from the findings of Chen et al.,<sup>1</sup> but also from similar observations in rhodopsin kinase (RK) knockout animals<sup>7</sup> and from the discovery of new forms of photoreceptor dystrophies for which constitutive activation of the visual cascade is the most likely explanation for degeneration. These include mutations of the rod  $\alpha$  subunit of the cyclic guanosine monophosphate (cGMP)-gated channel<sup>8</sup> and of the retinal guanylyl cyclase (RetGC-1).<sup>9,10</sup> Mutations of the channel leave the photoreceptor outer segment with almost no resting influx of  $\text{Na}^+$  or  $\text{Ca}^{2+}$ , much as if the receptor were constantly exposed to a very bright light (Fig. 1). Similarly, mutations of the cyclase prevent the synthesis of cGMP, resulting in a very low resting cGMP concentration and closure of the cGMP-gated channels. Thus, both these mutations lead to a situation in which the rod is continuously hyperpolarized in the dark, just as it would be during saturating continuous light.

This equivalent-light signal produced in the photoreceptors is apparently also signaled to other retinal cells. The best evidence for this comes from recent work in the chicken retina, where the pigment granules of the retinal pigment epithelium take a very different position when the animal is in the light or the dark. In chick *rd* animals in which one form of guanylyl cyclase (RetGC-1) is absent, the pigment granules remain in the "light" configuration, even when the animals are kept in darkness.<sup>11</sup> The photoreceptors in these animals are apparently sending a signal to the pigment epithelial cells that is equivalent to the one that occurs in light.

## Possible Mechanisms by Which Equivalent Light Might Produce Degeneration

In photoreceptors containing mutations for cGMP-gated channels or guanylyl cyclase, or in arrestin knockout mice, the transduction cascade is strongly activated. Because intracellular  $\text{Ca}$  ( $\text{Ca}^{2+}_i$ ) is heavily dependent on  $\text{Ca}^{2+}$  influx through the cGMP-gated channels, it appears very likely that  $\text{Ca}^{2+}_i$  will be low in these animals (Fig. 1). In the central nervous system,  $\text{Ca}^{2+}_i$  is known to play an important role in degeneration, particularly in cases for which  $\text{Ca}^{2+}_i$  is excessively elevated during stroke or trauma.<sup>12</sup> Elevated  $\text{Ca}^{2+}_i$  is thought to disrupt the membrane potential and outer membrane of the mitochondria and produce the release of cytochrome c and other proteins that activate caspases, which are proteases that mediate programmed cell death or apoptosis.<sup>13</sup> In the retina, elevated  $\text{Ca}^{2+}_i$  has long been thought to be responsible for some forms

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From the Departments of <sup>1</sup>Physiological Science and <sup>2</sup>Ophthalmology, University of California Los Angeles; and the <sup>3</sup>Department of Biology and Center for Complex Systems, Brandeis University, Waltham, Massachusetts.

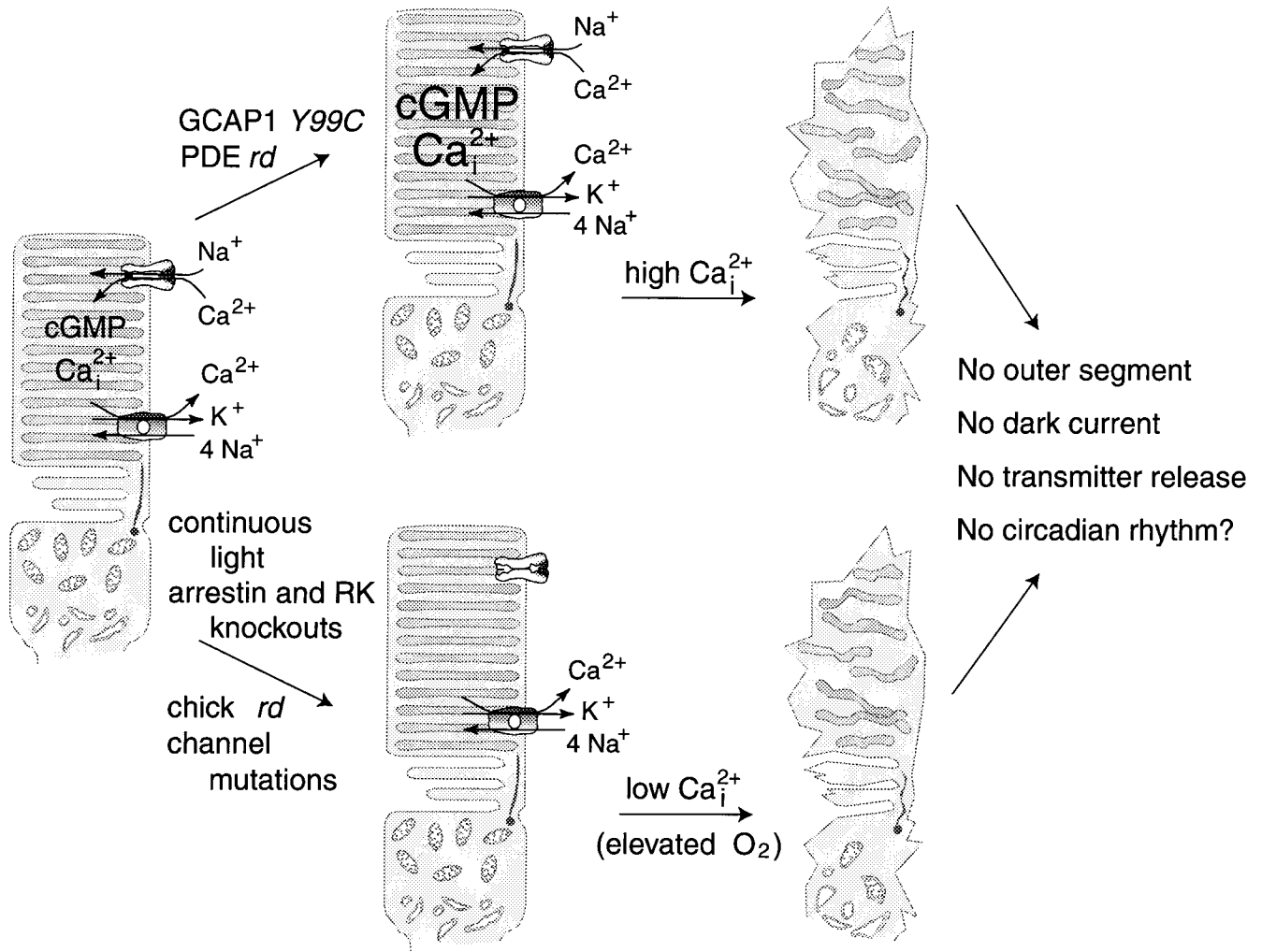
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Corresponding author: Gordon L. Fain, Department of Physiological Science, 3836 Life Sciences Building, University of California Los Angeles, Los Angeles, CA 90095-1527.

E-mail: gfain@ucla.edu



**FIGURE 1.** Equivalent light and mechanisms of degeneration. *Left:* a normal, dark-adapted rod outer segment, with  $\text{Na}^+$  and  $\text{Ca}^{2+}$  entering through cGMP-gated channels, and  $\text{Ca}^{2+}$  exported through the  $\text{Na}^+/\text{K}^+-\text{Ca}^{2+}$  transporter. The mutations *PDE rd* and *GCAP1 Y99C* both have been shown to produce high outer segment levels of cGMP that have been postulated to produce high intracellular  $\text{Ca}^{2+}$ , which may lead to degeneration (*top: middle and right*). Real or equivalent light produced by cyclase or channel mutations, or by knocking out the arrestin or rhodopsin kinase (RK) gene, would lead to low levels of cGMP and closed channels. Because  $\text{Ca}^{2+}$  entry is blocked, there is a low level of intracellular  $\text{Ca}^{2+}$  (*bottom: middle*). Either low  $\text{Ca}^{2+}$  or elevated  $\text{O}_2$  may then trigger apoptosis (*bottom: right*). The loss of the outer segment and eventual death of the photoreceptor would then eliminate the photoreceptor light response and block transmitter release, which may be responsible for spreading the equivalent-light signal to other cells, perhaps as the result of blocking the retinal circadian rhythm.<sup>5</sup>

of degeneration, for example in animals with the phosphodiesterase *rd*<sup>14</sup> or the *GCAP1 Y99C* mutations.<sup>15,16</sup>

More recent evidence indicates that too low a  $\text{Ca}^{2+}$  concentration also seems to produce cell death. Cultured neurons deprived of growth factors normally die but can be rescued in medium containing high  $\text{K}^+$ , which produces membrane depolarization.<sup>17</sup> This protection from death has been shown to be produced by the gating of  $\text{Ca}^{2+}$  channels, leading to an increase in the intracellular free  $\text{Ca}^{2+}$  concentration<sup>18,19</sup> and the activation of CaM-kinase kinase.<sup>20</sup> Apoptosis produced by low  $\text{Ca}_i^{2+}$  may be an important mechanism of cell death and synapse elimination in the nervous system during development, because neurons that are not depolarized by ongoing synaptic input would not receive sufficient stimulation to keep  $\text{Ca}_i^{2+}$  above a minimal level. A similar process may produce degeneration in photoreceptors whenever the  $\text{Ca}_i^{2+}$  is maintained at too low a level over a too prolonged a period.

This  $\text{Ca}^{2+}$  hypothesis is unlikely to be the only mechanism of photoreceptor death. Travis,<sup>21</sup> for example, has suggested

that many forms of degeneration, including those produced by real or equivalent light, may be mediated by  $\text{O}_2$  toxicity. Loss of the photoreceptor response may decrease  $\text{O}_2$  consumption in the outer retina enough to raise  $\text{O}_2$  tension to levels that may be toxic. Another possibility is that constant real or equivalent light may disrupt vital circadian processes.<sup>5</sup> Degeneration in some forms of retinitis pigmentosa may also be caused by disruption of the structure of the photoreceptor or abnormal transport of protein to the outer segment plasma membrane. It seems possible, however, that disruption of the outer segment plasma membrane may also disrupt the  $\text{Ca}^{2+}$  economy of the outer segment, perhaps by making the plasma membrane too leaky to  $\text{Ca}^{2+}$  or by inhibiting the synthesis of cGMP.

Some mutations that produce constitutive activation lead to stationary night blindness. It may be that humans with Oguchi disease<sup>3,22</sup> or with the rhodopsin G90D mutation<sup>23</sup> have a lowered outer segment  $\text{Ca}_i^{2+}$  during normal cyclic light exposure, but the  $\text{Ca}_i^{2+}$  concentration may not be low enough for a sufficiently long period to trigger rapid degeneration.

Now that  $Ca^{2+}_i$  measurements from mammalian photoreceptors are feasible,<sup>24</sup> it will be interesting to test possible correlations between  $Ca^{2+}_i$  and photoreceptor survival.

There is now considerable support for the equivalent-light hypothesis, and it might be asked what further experiment would provide a definitive proof. An important prediction of this hypothesis is that degeneration, produced, for example, in arrestin knockout or RK knockout mice by continuous illumination, should be prevented if a second mutation were introduced that blocked the transduction cascade. Thus, ironically, a mutation that "blinded" the rod should block the degeneration. Although this would clearly not be an advisable therapeutic strategy, the introduction of a second blinding mutation in arrestin knockout animals would be a definitive test of whether an equivalent-light signal is responsible for at least some forms of retinal degeneration.

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