Increased Susceptibility to Light Damage in an Arrestin Knockout Mouse Model of Oguchi Disease (Stationary Night Blindness)

Jeannie Chen, Melvin I. Simon, Michael T. Matthes, Douglas Yasumura, and Matthew M. LaVail

PURPOSE. To determine whether constitutive signal flow arising from defective rhodopsin shut-off causes photoreceptor cell death in arrestin knockout mice.

METHODS. The retinas of cyclic-light-reared, pigmented arrestin knockout mice and wild-type littermate control mice were examined histologically for photoreceptor cell loss from 100 days to 1 year of age. In separate experiments, to determine whether constant light would accelerate the degeneration in arrestin knockout mice, these animals and wild-type control mice were exposed for 1, 2, or 3 weeks to fluorescent light at an intensity of 115 to 150 fc. The degree of photoreceptor cell loss was quantified histologically by obtaining a mean outer nuclear layer thickness for each animal.

RESULTS. In arrestin knockout mice maintained in cyclic light, photoreceptor loss was evident at 100 days of age, and it became progressively more severe, with less than 50% of photoreceptors surviving at 1 year of age. The photoreceptor degeneration appeared to be caused by light, because when these mice were reared in the dark, the retinal structure was indistinguishable from normal. When exposed to constant light, the retinas of wild-type pigmented mice showed no light-induced damage, regardless of exposure duration. By contrast, the retinas of arrestin knockout mice showed rapid degeneration in constant light, with a loss of 30% of photoreceptors after 1 week of exposure and greater than 60% after 3 weeks of exposure.

CONCLUSIONS. The results indicate that constitutive signal flow due to arrestin knockout leads to photoreceptor degeneration. Excessive light accelerates the cell death process in pigmented arrestin knockout mice. Human patients with naturally occurring mutations that lead to nonfunctional arrestin and rhodopsin kinase have Oguchi disease, a form of stationary night blindness. The present findings suggest that such patients may be at greater risk of the damaging effects of light than those with other forms of retinal degeneration, and they provide an impetus to restrict excessive light exposure as a protective measure in patients with constitutive signal flow in phototransduction. (Invest Ophthalmol Vis Sci. 1999;40:2978–2982)

Naturally occurring mutations that lead to a constitutive signal flow in phototransduction have been characterized in rhodopsin, transducin, arrestin, and rhodopsin kinase genes. Constitutive signal flow in phototransduction is thought to underlie some forms of retinal disorders. The so-called equivalent-light hypothesis has been proposed by Fain and Lisman in which constitutive phototransduction signals are equivalent to continuous or excessive light exposure, ultimately leading to cell death. It has been proposed that some naturally occurring mutations leading to blindness in humans, such as the absence of the rod photoreceptor ion channel, vitamin A deficiency, and L296E mutations in rhodopsin, are consistent with the equivalent-light hypothesis because the effect of these mutations on phototransduction simulates light exposure. However, the progression of photoreceptor damage is difficult to track in human patients, and only the endpoint condition is typically documented. Because of this limitation and others (see the Discussion section), the equivalent-light hypothesis remains to be rigorously tested under controlled experimental conditions.

Unabated signal flow can arise from different steps in the visual cascade. For example, certain mutations in rhodopsin can lead to constitutive activity, especially those that affect the salt bridge between Lys-296 and Glu-113. The interaction between Lys-296 and Glu-113 constrains the chromophore-free opsin to an inactive conformation. Disruption of this bond leads to an opsin conformation that can support transducin activation. Two known naturally occurring mutations in hu-
mans, A292E and G90D, result in the disruption of this salt bridge by competing for the charged residues and are thought to be responsible for causing stationary night blindness.\textsuperscript{1-7} The night blindness is thought to arise from an inability of rods, the dim-light photoreceptors, to respond to actual light signals in the environment because of the dark-light signals persisting from the mutant opsin.\textsuperscript{8} In transducin, a mutation in the $\alpha$-subunit in a position homologous to the oncogene p21\textsuperscript{ras} is thought to lead to prolonged activity.\textsuperscript{5} This mutation is diagnosed in patients as the Nougaret form of congenital stationary night blindness.\textsuperscript{5}

Defects in rhodopsin shut-off can also lead to prolonged signal flow. Rhodopsin phosphorylation by rhodopsin kinase and subsequent binding of arrestin are necessary steps in the complete inactivation of the visual pigment. A recessive condition called Oguchi disease is diagnosed in patients with naturally occurring mutations that lead to nonfunctional arrestin and rhodopsin kinase.\textsuperscript{9,10} Similar to the rhodopsin A292E and G90D mutations, Oguchi disease is thought to be a type of stationary night blindness. The implication of this clinical diagnosis is that daytime vision remains unaffected throughout the patient's lifetime.

In light of recent reports that some patients with arrestin null mutations have retinitis pigmentosa,\textsuperscript{11} it is particularly relevant to evaluate whether constitutive signal flow due to defective rhodopsin shut-off can cause photoreceptor cell death. We had an opportunity to examine this issue using pigmented mice without arrestin that we generated using homologous recombination.\textsuperscript{12,13} We have previously demonstrated that the absence of arrestin leads to defective rhodopsin shut-off and subsequently to prolonged photoresponse.\textsuperscript{14} Because of this defect, the rods saturate at very low light intensities and require an excessively long time to recover to the dark-adapted state after light exposure.\textsuperscript{11} Thus, if the defective rhodopsin shut-off and prolonged photoresponse can lead to photoreceptor cell death, we would predict that the arrestin knockout mice would be damaged at light levels that have no effect on normal photoreceptors. We have now found this to be the case. The observations have important implications for human patients with such defects, such as those with Oguchi disease.

**Materials and Methods**

**Mice and Lighting**

The knockout allele was maintained in pigmented mice with a mixture of 129sv and C57BL/6 genetic backgrounds, the strains used as wild-type control mice. The wild-type and arrestin knockout mice were born and reared in the same cyclic lighting conditions in our laboratory (by MML), with a 12-hour light–12-hour dark cycle at an in-cage illuminance of less than 15 fc. Some mice were reared in the dark, and others that were cyclic-light reared to the age of postnatal day (P) 100 were exposed to constant fluorescent light at an intensity of 115 to 150 fc for periods of 1, 2, or 3 weeks, as described elsewhere.\textsuperscript{15}

**Retinal Histology and Morphometric Analysis**

The mice were killed by overdose of carbon dioxide inhalation and immediately perfused intracardially with a mixture of mixed aldehydes (2% paraformaldehyde and 2.5% glutaraldehyde). All procedures with the animals adhered to the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the University of California San Francisco Committee on Animal Research.

Eyes were removed and embedded in epoxy resin, and histologic sections were made along the vertical meridian.\textsuperscript{16} The tissue sections were aligned so that the rod outer segments and Müller cell processes crossing the inner plexiform layer were almost continuous throughout the plane of section to ensure that the sections were not oblique, and the thickness of the outer nuclear layer (ONL) was measured as described elsewhere.\textsuperscript{15} Fifty-four measurements of the ONL were made in 18 contiguous fields around the entire retinal section (three measurements per field). These 54 measurements were either averaged to provide a single value for each retina to allow statistical comparison of groups or plotted as a distribution across the retina.

**Results**

In cyclic light, the wild-type mice showed a normal appearance (Fig. 1a) and no significant change in the number of photoreceptor nuclei on the basis of ONL thickness, an index of photoreceptor number\textsuperscript{17} at all ages up to 1 year (Fig. 2). The arrestin knockout mice kept in cyclic light, in contrast, showed degenerative changes as early as P100, including shorter and more disorganized rod outer segments (Fig. 1b) than normal (Fig. 1a). The ONL thickness at P100 was already slightly reduced in thickness from that in normal, wild-type mice (Figs. 1a, 1b, 2). With increasing age, the degeneration and loss of photoreceptors in the cyclic light-reared arrestin knockout mice became progressively more severe (Fig. 2). By 1 year of age, the ONL in most of the mice was reduced to less than 50% of the normal number (Figs. 1c, 1d, 2). In each case, from P180 to P365 the degenerative changes were more severe in the inferior than in the superior hemisphere (Figs. 1c, 1d).

The photoreceptor degeneration in cyclic light appeared to be caused by light itself, because when these mice were reared in the dark, the retinal structure was indistinguishable from that in normal wild-type control animals (Figs. 1c, 2). It was concluded therefore that cyclic light causes a slow, progressive loss of photoreceptors in the arrestin knockout mice.

To determine whether constant light would accelerate the degeneration in arrestin knockout mice, these animals and wild-type control mice at the age of P100 were exposed for 1, 2, or 3 weeks to fluorescent light at an intensity of 115 to 150 fc. As expected from results in previous studies,\textsuperscript{12,13} the wild-type pigmented mice retained normal retinal structure with no degenerative changes or loss of photoreceptor nuclei (Figs. 1f, 2) regardless of the length of constant light exposure. The pigmented arrestin knockout mice showed rapid photoreceptor degeneration when exposed to constant light, with the reduction in ONL thickness of 50% after 1 week of exposure and greater than 60% after 3 weeks of exposure (Figs. 1g, 1h, 2). The loss of photoreceptors in the arrestin knockout mice was significantly greater in the inferior than in the superior hemispheres of the eye (Fig. 3).

**Discussion**

We have found that in pigmented arrestin knockout mice with defective rhodopsin shut-off and prolonged photoresponse.
photoreceptors were progressively lost when the animals were maintained in cyclic light. The fact that the degeneration was prevented when the knockout mice were reared in the dark indicates that the excessive signal flow was light mediated.

When the pigmented arrestin knockout mice were exposed to constant light, photoreceptor degeneration was markedly accelerated. The degree of light-induced damage in the pigmented arrestin knockout mice (Fig. 3) was almost identical with that seen in albino mice. Thus, the arrestin knockout results in a change in susceptibility of the retina to constant light that apparently negates the high level of protection normally afforded by eye pigmentation. The normal-pigmented control mice were undamaged for up to 3 weeks of constant light, as expected from results in previous studies in which similarly light-exposed pigmented mice showed no degeneration for up to 18 weeks or 23 weeks.

Another significant difference between light damage in the arrestin knockout mice and normal albino mice is that the arrestin knockout mice show a greater sensitivity to light in the inferior hemisphere (Fig. 3), whereas normal albino mice are more severely damaged in the superior hemisphere of the eye.

One explanation of the much greater susceptibility of the arrestin knockout mice to excessive light and the reversal in hemispheric sensitivity may lie in different degeneration mechanisms from those seen in normal albino animals usually used in constant light experiments. The main damaging agent in the nonpigmented albino eye is thought to be reactive oxygen species. However, it is unlikely that significant levels of free radicals were generated from the amount of light irradiating the retinas in the pigmented arrestin knockout mice, given that normal pigmented mice show no damage with up to 23 weeks of similar constant light exposure. Nevertheless, the amount of light entering the pigmented eye should be sufficient to generate a signal flow that might be matched only by bright-light exposures when normal shut-off is in place. Clearly, direct experimental evidence is needed to ascertain the levels of reactive oxygen species in the arrestin knockout mice, but our findings suggest that the arrestin mouse model can allow for a functional dissection of two molecular bases of pathogenesis: constitutive signal flow and free radical generation.

Certain experimental results appear to be in conflict with the equivalent-light hypothesis. For example, transgenic mice overexpressing rhodopsin that cannot be properly turned off by phosphorylation (Ser334ter) show photoreceptor cell loss independent of light exposure. Overexpression of Lys296Glu in photoreceptors of transgenic mice also causes retinal degeneration that is apparently not related to elevated rhodopsin activity, because it is inactivated by arrestin binding. It should be pointed out that these animal models were generated by a gene-addition technique in which the transgene is expressed in addition to the endogenous wild-type rhodopsin. Importantly, it has been observed that rhodopsin overdosage, itself, can be detrimental to photoreceptors. The carboxyl terminal of rhodopsin, furthermore, has been implicated in vectorial transport of rhodopsin in photoreceptors and polarized MDCK cells. Deletion of this domain can be expected to disrupt rhodopsin transport and adversely affect the health of photoreceptors through a mechanism that is unrelated to phototransduction. These confounding variables therefore interfere with the proper testing
of the equivalent-light hypothesis. In the arrestin knockout mice used in the present study, the only perturbation to the system was the removal of this capping protein, leading to a defined defect in phototransduction shut-off. Our results therefore provide strong support to the notion that constitutive signal flow is a stimulus for photoreceptor cell death. In other mice with a clearly defined defect in phototransduction shut-off—that is, in rhodopsin kinase knockout mice—constitutive signal flow appears to be a stimulus for photoreceptor cell death.35

It has been clearly shown in the normal rat retina that the superior hemisphere is damaged more severely by excessive light than the inferior hemisphere, regardless of the pigmentation type or direction of the light source.19,36 Thus, some undefined intrinsic difference exists in the two hemispheres of the rat retina in the response to constant light, and a similar increased susceptibility of the superior hemisphere exists in the mouse retina.15,21 The significantly increased susceptibility of the inferior hemisphere to constant light in the arrestin knockout mice also suggests that asymmetry exists in the substrate for the degeneration. This remains to be identified.

It is thought that cone photoreceptors are lost as a consequence of rod cell death.37,38 Because of this dependency of cones on rod survival, both daytime vision and nighttime vision are eventually lost, even when the primary defect lies in the rod photoreceptors. We provide evidence that rod photoreceptors die from constitutive signal flow that is light induced. The progression of this cell death may eventually lead to cone loss and subsequently to total blindness, as is evidenced in some patients with diagnosed Oguchi disease. However, we have now found that photoreceptor cell death can be prevented by removing the light stimulus in arrestin knockout mice. Our results therefore provide an incentive for restricting light exposure in those patients who have retinal disorders arising from constitutive signal flow.

There is accumulating evidence that photoreceptors undergoing inherited and age-related retinal degenerations may, in general, be more susceptible to the damaging effects of excessive light.9,59–42 The arrestin knockout mice, as far as we are aware, are the most sensitive to the damaging effects of light of any of the rodent models tested and are the first...
pigmented model to show progressive retinal degeneration due simply to cyclic light exposure. This underscores the notion that patients with mutations leading to nonfunctional arrestin and rhodopsin kinase, such as Oguchi disease, should avoid excessive light exposure.

References


42. LaVail MM, Gorsin GM, Yasumura D, Matthes MT. Increased susceptibility to constant light in nd and pcd mice with inherited retinal degenerations. *Invest Ophthalmol Vis Sci.* 1999;40:1020–1024.