Functional Abnormalities in Transgenic Mice Expressing a Mutant Rhodopsin Gene

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Purpose. To evaluate the consequences of the expression of a mutant mouse opsin gene on rod- and cone-mediated function. Experimental conditions were chosen to provide a basis of comparison to the results reported for patients with autosomal dominant retinitis pigmentosa (ADRP) in whom the proline at position 23 has been replaced by a histidine (P23H).

Methods. The mutated gene product resulted in three substitutions in the rhodopsin molecule: P23H, glycine for valine at position 20 (V20G), and leucine for proline at position 27 (P27L). Mice positive for the transgene were differentiated from normal littermates by the polymerase chain reaction. Electroretinograms (ERGs) were obtained from anesthetized mice between 1 and 9 months of age. After photically bleaching approximately 18% of the available rhodopsin, the time course of rod dark adaptation was examined by monitoring rod ERG amplitude recovery. Rhodopsin densitometry was used to determine the relative amounts of rhodopsin in the retina of normal and transgenic mice.

Results. ERGs obtained from transgenic mice showed a significant reduction in rod-mediated response amplitude at 1 month of age and a relatively slow progressive decrease thereafter. Cone-mediated ERGs, on the other hand, were nearly normal in amplitude for approximately the first 5 months after birth, but at later ages response amplitudes also underwent a progressive decline. In the normal retina, rod ERG amplitudes returned to prebleach levels within 30 minutes, whereas in transgenic mice response amplitudes did not recover within a 2-hour test period. The age-related decline in rod-mediated electroretinal potentials seen in transgenic mice was paralleled by a concomitant fall in rhodopsin density, and the sensitivity losses obtained electroretinographically could be accounted for solely on the basis of reduced quantal absorption.

Conclusions. The pattern of functional changes seen in the transgenic mice are in good agreement with those reported in patients with ADRP with the P23H mutation in the rhodopsin gene. Particularly noteworthy is the fact that the changes in rhodopsin density and visual sensitivity are associated with a progressive shortening of the rod outer segments; the histologic changes induced by the disease process in patients with ADRP have yet to be determined.


Genomic analysis of patients with autosomal dominant retinitis pigmentosa (ADRP) has provided convincing evidence that various subtypes of ADRP can result from mutations in the gene encoding rod opsin, a protein that binds 11-cis-retinal to form the visual pigment, rhodopsin. The genetic defect results in point mutations in one or another of the amino acids that constitute the polypeptide chain in the disk membranes of the rod outer segments and leads ultimately to the loss of visual cells and blindness.

Transgenic mice with rhodopsin mutations at comparable sites exhibit disorders of a similar nature. Recently, Naash et al described a slow retinal degeneration in transgenic mice expressing an opsin gene with three point mutations within a seven amino acid sequence near the N-terminus. One of these mutations involves the replacement of proline with histidine at position 23 (P23H), a substitution that occurs also in human patients with ADRP. The other two mutations, substituting glycine for valine at position 20 (V20G) and leucine for proline at position 27 (P27L), have not been associated with
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human ADRP, and they were included to enhance antibody recognition of the mutant rhodopsin and differentiation of normal and transgenic mice. Animals expressing the mutated opsin gene will be referred to as VPP mice. These mice display retinal abnormalities that mimic in two important respects the features of human ADRP in which the P23H mutation has been implicated: (i) the degenerative changes involve both rod and cone photoreceptors, with a concomitant decline in the magnitude of the light-evoked electroretinogram (ERG), and (ii) the visual loss progresses at a comparatively slow rate; visual function, measured electroretinographically, is retained for several months, a relatively long period in the life of animals whose longevity rarely exceeds 2 years. In addition to these functional aberrations, earlier studies have demonstrated an age-dependent reduction in the length of the rod outer segments and a decrease in the cellular content of the outer nuclear layer.

In the present study, we provide additional evidence that the transgenic mice mimic the human condition in terms of the relative losses of rod- and cone-mediated function and the time course of recovery of rod-mediated function after exposure to a bright, light-adapting field. In addition, we have attempted to address more directly the putative mechanisms that link the gene mutation with the pathogenesis of the degenerative process. One interesting hypothesis, put forth recently to account for the progressive loss of visual cells and the decline in photic sensitivity, suggests that the mutation in some forms of retinitis pigmentosa (RP) may lead to the formation of a constitutively active rhodopsin, that is, a molecular species that continuously activates the transduction cascade and presumably induces pathologic changes that mimic the toxic effects of prolonged light exposure. To examine this possibility in VPP mice, we analyzed the electroretinographic responses and rhodopsin content of transgenic animals relative to that of normal littermates. Our results do not provide support for the notion that the opsin mutation leads to a constitutively active rhodopsin in this transgenic model of ADRP. Rather, sensitivity losses in the dark-adapted retina can be attributed to reduced quantal absorption by rhodopsin, a consequence of the reduction in the length of the rod outer segments and the concomitant loss of visual cells.

MATERIALS AND METHODS

Transgenic Mice

As described previously, the transgene was constructed by oligonucleotide-directed mutagenesis and insertion of a mutated fragment into lambda MO1 comprising the complete murine opsin gene. The final transgene consisted of a 15-kb mouse opsin genomic fragment that contained 6-kb upstream and 3.5-kb downstream sequences, and in which a part of the wild type exon 1 of the opsin gene was replaced by a fragment containing the mutations. Transgenic mice were identified by the presence of the restriction fragment length polymorphism that was created in the transgene. Polymerase chain reaction amplification of exon 1 with primers W75/W11, followed by digestion with NcoI, reveals three fragments in wild-type mice of 689 bp, 431 bp, and 197 bp. Transgenic mice have an additional (689 bp + 197 bp) fragment due to the deletion of the NcoI site. The retinal degeneration is inherited as a dominant trait in a Mendelian fashion. Therefore, in the heterozygote to normal matings used to generate the mice studied here, ~50% of the offspring are transgenic and ~50% are normal. Each mouse was tested without the experimenter knowing if the mouse was transgenic or normal. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Electroretinography

Mice were anesthetized with an intraperitoneal injection of 15 μl/g body weight of a saline solution containing ketamine (1 mg/ml), xylazine (0.4 mg/ml), and urethane (40 mg/ml). The pupil was dilated with 2.5% phenylephrine HCl, and the animals were placed on a heating pad. Electroretinograms were recorded using a coated stainless steel wire contacting the anesthetized (1% proparacaine HCl) corneal surface through a layer of 1% methylcellulose. A similar wire placed in the mouth and a needle electrode inserted in the tail served as reference and ground leads, respectively. Responses were differentially amplified (half bandpass: 1 to 1000 Hz), averaged, and stored using a Nicolet (Madison, WI) Pathfinder II signal averaging system. Strobe flash stimuli (t < 1 msec) were presented in a Nicolet ganzfeld (GS-2000), either in the dark or superimposed on an adapting field.

Electroretinograms were recorded in two sessions. In the first, dark-adapted, luminance-response functions were obtained using flash luminances ranging from −5.18 to 0.85 log cd-sec/m². Stimuli were presented in order of increasing luminance, and the responses to two successive flashes at each intensity were averaged. A 30-second interflash interval was used for the five lowest luminance flashes; a 1-minute interval was used for the eight higher luminances. The animals were then exposed to a 1.60 log cd/m² light-adapting field, and, after a period of 10 minutes during which responses reached a stable amplitude, ERGs were elicited at flash luminances ranging from −0.23 to 0.85 log cd-sec/m². At each luminance, responses to 50 successive flashes presented at a rate of 2.3 Hz were averaged.

In the second session, ERG recordings were ob-
RESULTS

Figure 1 shows representative ERG recordings from a 2-month-old normal mouse and from VPP mice of various ages in response to flash stimuli delivered under dark-adapted conditions (Fig. 1A) and presented on a steady background field of 1.60 log cd/m² (Fig. 1B). Flash luminance was the maximum available (0.85 log cd-sec/m²). It is evident that the amplitudes of the dark-adapted responses from transgenic animals were reduced as early as 1 month after birth and that there was a further reduction in the recordings from older animals. The light-adapted recordings from VPP mice gave a somewhat different picture (Fig. 1B). Response amplitudes appeared to be relatively unaffected until approximately 5 months of age and declined thereafter; by 9 months of age, however, the light-adapted response also was markedly reduced.

Rod and Cone Contributions to the ERG Waveform

There is good evidence to indicate that mouse ERGs evoked by incremental flashes superimposed on a background luminance of 1.60 log cd/m² (Fig. 1B) are mediated by the cone mechanism, but the relative contributions of the rod and cone systems to the dark-adapted potentials are not immediately apparent. Figure 2 shows results that address this question experimentally. In Figure 2A, the test flash was delivered first to the dark-adapted eye (dashed lines) and then on a background field (0.91 log cd/m², continuous lines) that exerted a strong suppressive effect on rod-mediated responses but had little effect on cone-mediated potentials. The recordings from a normal (N) and a 3-month-old transgenic (VPP) mouse indicate that the cone-mediated receptor potential makes only a negligible contribution to the dark-adapted a-wave in normal and transgenic mice. However, there is a substantial cone contribution to the dark-adapted b-wave recorded from the normal retina, and this component becomes even more prominent in the dark-adapted responses of the VPP mice owing to the profound loss of rod-mediated activity. On the other hand, when a dim test flash was presented to the dark-adapted eye, a b-wave-dominated response was elicited (Fig. 2B, dashed line); in this case, the test flash was reduced by almost 3 log units (to −2.12 log cd-sec/m²) as compared with that of Figure 2A. More important, no response was detectable to the same test flash when superimposed on the weak adapting field (Fig. 2B, solid line), indicating that only rod-mediated responses are activated at this stimulus intensity.

Progressive Changes in the Electroretinal Potentials

The age-related changes in dark- and light-adapted ERG components of transgenic mice are illustrated in Figures 2C and 2D, respectively. As mentioned earlier, a significant reduction in the ERG amplitude was already evident in the dark-adapted responses of VPP mice at 1 month of age (Fig. 2C). Both the a- and b-
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FIGURE 1. Electroretinograms obtained from representative normal and VPP mice of different ages to a 0.85 log cd-sec/m² flash stimulus (A) presented to the dark-adapted eye, or (B) superimposed on a background field of 1.60 log cd/m². Vertical dashed lines indicate stimulus onset. The amplitude of the a-wave was measured from the prestimulus baseline to the trough of the cornea-negative peak (a). The b-wave was measured from the a-wave trough to the positive peak (b); b-wave implicit times (see Fig. 4) represent the interval from flash onset to the b-wave peak.

wave potentials were approximately one half of normal at this stage and declined progressively in the responses from older animals. By 9 months of age, the a-wave was no longer detectable with the recording methods used here, but the b-wave was still in evidence. The apparent retention of the dark-adapted b-wave probably reflects the greater photic sensitivity of this response component as well as an underlying contribution from the cone-mediated b-wave potential (Fig. 2A).

Responses recorded under light-adapted conditions are derived almost exclusively from cone-mediated activity and, as shown in Figure 2D, exhibited very different age-related defects. The b-wave responses were only minimally reduced for the first 5 months after birth, and, although there was a gradual decline thereafter, response amplitudes recorded from 9-month-old animals were still about one third of normal at this relatively advanced age.

The Temporal Course of Dark Adaptation

Figure 3 illustrates the time-dependent changes in the ERG b-wave after exposure to a preadapting light (2.4 log cd/m²) that bleaches approximately 18% of the available rhodopsin in a normal retina. All responses were elicited with a constant test luminance (~2.12 log cd-sec/m²); note that with this intensity test flash (see Fig. 2B), only the rod system was activated throughout the course of dark adaptation. To minimize intersubject differences in absolute amplitude, responses for each mouse are expressed as a percentage of the average dark-adapted baseline for that mouse. After extinguishing the light-adapting field, normal mice (open circles) required approximately 10 minutes for amplitude to recover one half its original magnitude and approximately 25 to 30 minutes in darkness to return to the initial dark-adapted level. In normal mice, the response amplitudes overshot the original baseline values. This could be a property of the normal retina or may indicate that the retina had not been fully dark adapted at the onset of light adaptation. Data from the VPP mice (filled circles) show that the recovery of response amplitude during dark adaptation was grossly abnormal. The rate of recovery was retarded (t₁/₂ = 40 minutes), and response amplitudes did not reach the preexposure level within a 2-hour period of dark adaptation.

Rhodopsin and Retinal Sensitivity

Table 1 presents data on the rhodopsin density measurements and a-wave amplitude recordings from normal animals and VPP mice at 1 and 2 months of age. Rhodopsin values represent absorbance measurements (ΔD₅₀) at the peak of the difference spectrum (λ = 510 nm; see Fig. 4B). Note that at comparable ages, the two metrics are reduced relative to the normal by approximately the same proportion. Thus, at 1 month of age, the values for ΔD₅₀ and for the a-wave amplitude are reduced to 45% and 46% of normal, respectively, whereas at 2 months, the corresponding
FIGURE 2. (A) Electroretinographic recordings from a normal (N) and a VPP mouse to a 0.85 log cd-sec/m² flash stimulus presented in the dark (dotted lines) or against a 0.91 log cd/m² adapting field (continuous lines). Vertical dashed lines indicate the time of flash presentation. (B) Responses from a normal mouse elicited with the dim flash used to monitor dark adaptation (~2.12 log cd-sec/m²) presented under the same adaptation conditions as in (A). Note the absence of an underlying cone component in both the dark- and light-adapted responses. (C) Age-related changes in the amplitude of the dark-adapted ERG a-wave (circles) and b-wave (triangles). Open symbols represent the mean of 28 normal mice. Seven to 10 VPP mice were tested at 1 to 4 months; three to four VPP mice were tested at later ages. Error bars indicate ±1 SEM and are omitted when smaller than the plotted point. (D) Age-related changes in the amplitude of the cone electroretinographic b-wave; symbols as in (C).

FIGURE 3. Rod b-wave amplitude recovery during dark adaptation after the termination of a 2.4 log cd/m² background field; all responses were elicited with a constant flash luminance of ~2.12 log cd-sec/m². Amplitudes obtained from each mouse are normalized to the average baseline response obtained after dark adaptation. Open circles represent averaged (±1 SEM) results obtained from six normal 2-month-old mice. Filled circles indicate results obtained from four 2-month-old VPP mice.

reductions are to 32% and 25% of normal. As shown in Figure 4A, this relation applies also when retinal thresholds, derived from ERG measurements, are plotted as a function of the rhodopsin density, expressed as percent of normal. Three different indices of threshold were used: (i) the luminance required to generate a criterion a-wave response of 50 μV, (ii) the luminance giving rise to a 100 μV b-wave, and (iii) the luminance required to evoke a b-wave with an implicit time of 80 msec (a point that falls on the linear portion of the function relating b-wave implicit time to stimulus luminance; see Fig. 4C). The data for each of these threshold measures is approximated by a curve (solid line) that relates threshold elevations to a decrease in the probability of quantal absorption by rhodopsin. This so-called “probability function” has been shown to hold in patients with various forms of RP,26,27 and for conditions in which the rhodopsin density measurements are low due to a reduced length of the rod outer segments, for example, in the developing retina.21 The dashed line shows results obtained in rat, and represents the log-linear relation between b-wave threshold and rhodopsin density that applies to situations in which a fraction of the native rhodopsin is
TABLE 1. Rhodopsin Density and Maximum a-Wave Amplitudes of Normal and Transgenic Mice

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Rhodopsin ( \Delta D_{310} )</th>
<th>ERG a-wave (( \mu V ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>0.152 ± 0.011</td>
</tr>
<tr>
<td>VPP 1 month</td>
<td>6</td>
<td>0.070 ± 0.005</td>
</tr>
<tr>
<td>VPP 2 month</td>
<td>6</td>
<td>0.048 ± 0.008</td>
</tr>
</tbody>
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ERGs were recorded from mice that were dark adapted overnight; a-wave data are for responses to a 0.85 log cd·sec/m² flash stimulus. One to 2 days later, the same mice were again dark adapted and killed, and the retinas were isolated for rhodopsin densitometry. Tabulated values give the mean ± SEM.

depleted either by bleaching or as a consequence of vitamin A deficiency. It is clear that the data obtained from the VPP mice are not fit well by the log-linear relation.

DISCUSSION

The main objectives of the present study were to examine the effects on retinal function of the expression of the VPP point mutations in the murine rhodopsin gene and to assess some of the factors that might be responsible for the genetically induced abnormalities in structure and function. These mutations result in a slow degeneration of the rod photoreceptors, as demonstrated both histologically and by the gradual reduction in a-wave amplitude of the rod-mediated ERG (Figs. 1A, 2C). Moreover, it appears that the VPP mutations exert an effect on the development of the rod photoreceptors. Although the inner retina develops normally and the outer nuclear layer appears initially to contain a normal complement of photoreceptor nuclei, the rod outer segments never achieve the length seen in age-matched normal mice and they become progressively shorter as the disease progresses. This is consistent with the observation that the rod receptor potential (a-wave) is reduced by ~50% at the earliest age (1 month) at which the ERG was tested (Fig. 1A) and declines as a function of age (Fig. 2C). Because the transgene is expressed only in rods, the VPP mutations would be expected to spare initially the cone system, and the electroretinographic results indicate that this is indeed the case. The cone-mediated ERG amplitudes remained relatively normal until approximately 5 months of age, and only then did they begin to decrease (Figs. 1B, 2D). This observation suggests that cone dysfunction occurs subsequent to rod degeneration in VPP mice and reflects perhaps a structural disorganization of cone photoreceptors due to the loss of support provided by neighboring rods, or to the effects of some toxic by-product of rod degeneration. In any case, the factors underlying the various anomalies in the cone ERG remain to be identified.

Abnormalities in the recovery of ERG amplitude during dark adaptation (Fig. 5) provide a potentially important insight into the nature of the disease process. It is not possible with noninvasive techniques to identify the molecular basis for the remarkably slow time course over which dark adaptation proceeds in the VPP mice. Nevertheless, it is noteworthy that similar aberrations have been reported for psychophysical measures of dark adaptation in patients who have ADRP with the P23H rhodopsin mutation, as well as in some individuals with RP involving a different rhodopsin mutation or in whom genetic tests were not performed. There are obviously a number of possible explanations to account for this type of anomaly. The delay could reflect a defect in the processes underlying the regeneration of rhodopsin. For example, the VPP mutations might interfere with the process by which rhodopsin is reformed after bleaching. The binding site for the chromophore is located within the transmembrane domain of the opsin molecule, but there is evidence to suggest that the protein-chromophore interaction may be modified by changes in the intradiskal regions of the molecule, including the N-terminus. Thus, there is the possibility that the VPP mutations interfere with the mechanism by which 11-cisretinaldehyde enters the binding pocket of the mutant opsin. Alternatively, a delay in dark adaptation could be caused by prolonged lifetime of phototransduction byproducts that desensitize the rods. Photoreactivated rhodopsin (R') is normally inactivated by a sequence of events involving phosphorylation and arrestin binding. Any interference with these interactions by the mutated opsin molecules might produce delays in the recovery of visual sensitivity. However, each of the foregoing hypothetical scenarios implicates processes initiated by the photoreactivation of rhodopsin, and there is reason to doubt that these types of abnormalities are instrumental in triggering the disease process. The results of recent studies (Naash et al, manuscript in preparation) have shown that VPP mice reared in darkness from birth undergo a progressive, albeit slower, retinal degeneration similar to that seen in animals reared in cyclic lighting. Determining the mechanism underlying the delayed dark adaptation observed in VPP mice and in patients with the P23H form of ADRP is clearly a significant question for future studies.

There remains, of course, the possibility that the mutant rhodopsin of VPP mice is a constitutively active molecule that gives rise to a persistent 'dark light.' As mentioned earlier, it has been suggested that sustained activation of the transduction process may induce degenerative changes similar to those produced by prolonged exposure to light itself. Interestingly, there are a number of point mutations that re-
suit in opsins capable of constitutively activating transducin when expressed in an in vitro assay system. However, the presence of these mutations in humans results in widely disparate disorders, ranging from congenital stationary night blindness to a rapidly progressive form of RP. Neither of these conditions is similar in their functional consequences to those expressed by the VPP mice or patients who have ADRP with the P23H mutation in rhodopsin, nor has it been shown unequivocally that under light conditions that lead to photic injury, the activation of transducin per se is the event that induces the degenerative process.

Also inconsistent with the equivalent light hypothesis are the results shown in Figure 4, wherein log thresholds (the logarithm of the luminance required to achieve a criterion response) are plotted as a function of the rhodopsin content of the retina (expressed on a linear scale). Studies on a variety of vertebrate species, including humans, have shown that denuding opsin of its chromophore, whether by photic bleaching or vitamin A deprivation, leads to a disproportionately large loss of visual sensitivity relative to that expected simply from the decrease in quantal absorption. For reasons that are still unclear, the results seem to be best approximated by a log-linear relationship; b-wave data from rat provide a good example of this sort of relation (dashed line, Fig. 4A), although the slope of the function may differ for other species. The results obtained for the VPP mice fail to fit a pattern consistent with the equivalent light hypothesis. The data points of Figure 4 do not deviate significantly from the continuous curve that describes the function to be expected if the probability of quantal absorption were the sole determinant of the rise in threshold. In these circumstances, reducing the rhodopsin content of the rods by 50% would produce a twofold (0.3 log unit) rise in threshold, whereas a reduction of 90% would elevate threshold by only tenfold (1 log unit). This is consistent with the short rod outer segments of VPP mice seen histologically.

**FIGURE 4.** (A) The relation between electrically recorded thresholds (1/sensitivity) and the relative content of rhodopsin in normal (open symbols) and transgenic (filled symbols) mice. Three threshold criteria were used: the intensity required to elicit a 50-μV a-wave (circles), a 100-μV b-wave (diamonds), or an 80-msec b-wave (triangle). Data points for normal mice (open symbols) indicate the mean (±1 SEM; N = 7); data points for VPP mice represent the averaged (±1 SEM) value for four mice with similar rhodopsin densities, or the value obtained from a 7.5-month-old mouse with a low rhodopsin density (data at far right). Values for the three measures have been adjusted vertically in order that the normal means coincide. The solid line is the function expected if threshold elevation is due solely to a reduction in quantal absorption. The dashed line represents changes in b-wave threshold recorded during the regeneration of bleached rhodopsin in the rat retina. (B) Examples of the absorbance difference spectra (DDi) obtained from a normal mouse (open circles) and from VPP mice at different ages (1 month, filled circles; 2 months, filled triangles; 3 months, filled diamonds). (C) Examples of b-wave implicit time functions. The mean normal function is represented by open circles; individual VPP mice are shown as filled symbols. The solid lines represent the least-squares fit linear regression to the data spanning the linear range of each function. The dashed line indicates the 80-msec criterion from which the threshold luminance was determined.
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and with the ERG evidence that these photoreceptors are capable of functioning surprisingly well with this degree of structural impairment.10

Although it seems difficult to reconcile the retention of rod-mediated electrical activity with such gross losses in the rhodopsin content of the visual cells, it should be noted that similar findings have been reported in individuals with the P23H rhodopsin mutation,19 as well as in other patients with ADRP in whom the gene mutations were not identified.19,26,27 This situation, and the observation that the rod outer segments of VPP mice become progressively shorter with age,10 raise the possibility that one consequence of the opsin mutation is an imbalance between the rates of disk formation and disk shedding.19,26 The mutant opsin may induce a more rapid rate of disk shedding, or it may somehow impede the processes by which newly formed opsin is transported to, or inserted into, the disk membranes. In either event, the delicate balance between new disk formation and disk shedding is upset, and there follows a progressive shortening and eventual death of the visual cells.

It is important to recall that the rhodopsin expressed in the VPP mice includes three point mutations: V20G, P23H, and P27L. Although P23H is responsible for some forms of human ADRP,1,8,12 neither V20G nor P27L have been identified in patients with ADRP.20 This observation, and the fact that proline 23 is highly conserved among normal opsins in a wide variety of species,21 make it highly likely that the P23H mutation is primarily responsible for the retinal degeneration seen in the VPP mice. Nevertheless, it would be circumspect to determine whether mutations V20G, P27L, or both, contribute in some way to the degenerative process; lines expressing only one or the other of these mutations are necessary to address this possibility. Another fundamental issue that will be important for our understanding of the morphologic and physiological abnormalities observed in the transgenic animals concerns the functional status of the opsin induced by the VPP mutation. At present, we have no evidence as to whether the mutant opsin expressed in the VPP mice is capable of binding retinoid and interacting with the enzymes of the transduction cascade, or indeed whether it is inserted into the rod disk membranes.22,25 These questions are currently under investigation, and the outcome will obviously influence the interpretation of the data presented in this study.

These issues aside, the results of our experiments have identified several important functional similarities between the VPP mice and human subjects with the P23H form of ADRP. Individuals with the P23H mutation exhibit a relatively slow rod degeneration with initial sparing of cone function,1,2 an unusually slow rate of dark-adaptation,13 and threshold elevations that can be ascribed solely to the reduced probability of quantal absorption resulting from the diminished rhodopsin content of the rods.15 The results of the present study indicate that each of these characteristics is shared by the VPP mice. In view of the decades-long time course of human ADRP, the VPP mice appear to provide a useful animal model in which to examine the mechanisms by which mutations in the N-terminus region of rhodopsin disrupt the functional and structural integrity of the visual cells and lead ultimately to a widespread retinal degeneration.

Key Words

retinitis pigmentosa, electroretinogram, visual sensitivity, rhodopsin density, transgenic mice

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References

10. Naash MI, Hollyfield JG, Al-Ubaidi MR, Baehr W. Sim-


