Variation in Rhodopsin Kinase Expression Alters the Dim Flash Response Shut Off and the Light Adaptation in Rod Photoreceptors

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PURPOSE. Rod photoreceptors are exquisitely sensitive light detectors that function in dim light. The timely inactivation of their light responses is critical for the ability of rods to reliably detect and count photons. A key step in the inactivation of the rod transduction is the phosphorylation of the rod visual pigment, rhodopsin, catalyzed by G-protein-dependent receptor kinase 1 (GRK1). Absence of GRK1 greatly prolongs the photoreceptors’ light response and enhances their susceptibility to degeneration. This study examined the light responses from mouse rods expressing various levels of GRK1 to evaluate how their function is modulated by rhodopsin inactivation.

METHODS. Transretinal and single-cell rod electrophysiological recordings were obtained from several strains of mice expressing GRK1 at 0.3- to 3-fold the wild-type levels. The effect of GRK1 expression level on the function of mouse rods was examined in darkness and during background adaptation.

RESULTS. Altering the expression of GRK1 from 0.3- to 3-fold that in wild-type rods had little effect on the single photon response amplitude. Notably, increasing the expression level of GRK1 accelerated the dim flash response shut off but had no effect on the saturated response shut off. Additionally, GRK1 excess abolished the accelerated of saturated responses shut off during light adaptation.

CONCLUSIONS. These results demonstrate that rhodopsin inactivation can modulate the kinetics of recovery from dim light stimulation. More importantly, the ratio of rhodopsin kinase to its modulator recoverin appears critical for the proper adaptation of rods and the acceleration of their response shut off in background light. (Invest Ophthal Vis Sci. 2011;52:6793–6800) DOI:10.1167/iovs.11-7158

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METHODS

Animals

Transgenic mice were generated, genotyped, and maintained on the C57BL6 background as previously described. All experimental protocols were in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committees at Schepens Eye Research Institute, Washington University, State University of New York at Buffalo, and Roswell Park Cancer Institute.

Analysis of Expression Levels

Quantitative RT-PCR and immunoblot analysis were carried out as previously described to estimate the levels of GRK1 in overexpressing mice. Briefly, total RNA and protein fractions were isolated from whole globes or retinas using the Trizol method. RNA samples from each line were reverse transcribed, and the amplification of a Grk1 cDNA was primed and monitored in real time in the presence of SYBR green as previously described to determine the cycle to detection threshold (Ct). Comparison of the Ct values or those corrected for the levels of actin, or beta 2 microglobulin (ΔCt) served as the basis for the calculation of the relative Grk1 RNA content across the samples. The reverse transcription and amplifications were performed in triplicate using RNA of four individual animals per strain.

For protein blotting, Trizol pellets were solubilized in isoelectric focusing buffer or and applied to gel at serial dilutions for immunoblot analysis and subsequent chemiluminescent signal detection as previously described. Monoclonal GRK1 antibodies (G8 or D11; Abcam, Boston, MA), polyclonal anti-recoverin (kindly provided by Alexander M. Dizhoor, Salus University) and RGS9-1 antibody (kindly provided by Ching-Kang Jason Chen, Virginia Commonwealth University) or anti-mouse beta actin (Abcam) were used as primary antibodies. Actin band signal served as the internal standard against which the signal for GRK1 band was normalized before comparison across samples using the previously described algorithm. At least four different pools of eyes were examined per strain to determine the relative levels of expression.

Immunostaining

Cryosections were taken of the eyes from various mouse strains and stained with a combination of peanut neuroagglutinin (PNA) and GRK1 polyclonal antibody 8585 as previously described.

Light Stimulation

For both transretinal and single-cell recordings, test flashes at 500 nm were delivered from a calibrated light source via computer-controlled shutters. Flash intensity was set by a combination of neutral density filters. Calibrated 520-nm light was used for background stimulation.

Transretinal Recordings

Mice were maintained in 12/12 hour light/dark cycle and dark-adapted overnight before experiments. After euthanatization, the eyes were removed under dim red light. A quarter of the isolated retina was mounted on filter paper photoreceptor-side up and placed on the recording chamber with an electrode connected to the bottom. A reference electrode was placed above the retina. The perfusion Locke solution contained 112 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl2, 1.2 mM CaCl2, 10 mM HEPES, 20 mM NaHCO3, 3 mM Na2-succinate, 0.5 mM Na-glutamate, 10 mM glucose, and was equilibrated with 95% O2/5% CO2, pH 7.4. Membrane current was recorded from the outer segment of a single rod photoreceptor protruding from the retina and drawn into the electrode. The suction electrode was filled with solution containing 140 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl2, 1.2 mM CaCl2, 3 mM HEPES, 10 mM glucose, pH 7.4. Responses were amplified by a current-to-voltage converter (Axpochip 200B; Molecular Devices, Sunnyvale, CA), low-pass filtered by an eight-pole Bessel filter with a cut off frequency of 30 Hz (Model 3382; Krohn-Hite), digitized at 1 kHz and stored on a computer using acquisition software (pClamp 8.2; Molecular Devices).

The kinetics of dim flash responses were determined as follows: time to peak was measured from the mid-point of the test flash; integration time was estimated as the time integral of the dim flash response after normalization of its amplitude to unity; recovery time constant (τrec) was estimated from a single exponential fit to the second half of the response shut off phase. Finally, the dominant time constant (τs) was determined from the slope of the time in saturation versus flash intensity plot for saturated flash responses. Rod sensitivity was monitored from the shift in the flash intensity required to produce half maximal response (I0). The rod single photon response was estimated by dividing the amplitude of a dim flash response by the flash intensity and the rod collecting area 0.5 μm2. We used the two-tailed unpaired Student’s t-test to determine the significance of difference unless otherwise noted.

RESULTS

The Expression Level of Rhodopsin Kinase in Mouse Rods Can Be Modulated over a 10-Fold Range

We generated several transgenic mouse lines with varying levels of rhodopsin kinase (GRK1) expression to study the impact of rhodopsin phosphorylation on the rod light response shut off. The lines carried copies of a bacterial artificial chromosome (BAC) construct in their genome containing the mouse Grk1 gene along with the entire 5’ and 3’ flanking sequences. Of the three lines with the full transgene, lines 1 and 3 designated Grk1+ and Grk1+b overexpressed GRK1 based on quantitative RT-PCR and immunoblot-based criteria (Figs. 1A and 1B), whereas line 2 (Grk1+a) did not overexpress GRK1 (Fig. 1A). In Grk1+b and Grk1+ lines, expressions were at 200% and 300% of the WT background, respectively (see also Ref. 12). The retinas overexpressing GRK1 showed no evidence of degeneration at least until 18 months of age. Retinal cryosections colabeled with antibodies recognizing GRK1 and the cone specific marker peanut agglutinin (PNA) showed remarkably uniform expression of GRK1 in rods and cones in both lines (Fig. 1C). Because of the higher kinase expression levels of the Grk1+ line and its markedly greater fertility, our studies focused primarily on this line. To expand the range of GRK1 expression levels, we also used heterozygous Grk1 knockout mice (Grk1+/−), with rods expressing GRK1 at 30% of WT rods as previously quantified. Together, the Grk1+/− and Grk1+ animals gave us a 10-fold range of GRK1 expression levels, from 0 to 3-fold the WT levels. Alterations in Grk1 expression did not affect the levels of other key molecules involved in regulation of recovery including recoverin, RGS9, or arrestin as seen in Figure 1D and as documented previously.
Overexpression of Rhodopsin Kinase Modulates the Overall Rod Retina Response

We initially compared the light responses of Grk1+/H11001 retinas with those of WT retinas to evaluate the functional impact of increasing GRK1 on rod function. Figures 2A and 2B show representative families of transretinal flash responses obtained from isolated dark-adapted wild-type (WT) and GRK1+ retinas. These traces were obtained under conditions that pharmacologically suppress synaptic transmission and postsynaptic ac-

FIGURE 2. Typical transretinal flash response families from (A) WT and (B) GRK1 overexpressing (Grk1+) retinas evoked by 500-nm flashes of 20 ms duration. Flashes of incremental intensities, in 0.5 log steps, were delivered at time 0. The dimmest flashes delivered 27 photons μm⁻². (C) Averaged normalized rod dim flash responses for WT (black; n = 8) and Grk1+ (gray; n = 6) retinas.
tivity (see Methods). As a result, the light-induced electrical deflections reflect the en mass rod activity with minimal contribution from second-order neurons or cones. As seen in Table 1, the mean rod sensitivities and response amplitudes across the strains remained statistically indistinguishable based on comparison of the intensities required to produce half maximal response \((I_0)\) and the maximal response amplitudes \(R_{max}\). These results are consistent with our morphologic studies and demonstrate that increasing by threefold the expression level of GRK1 in mouse rods from animals raised in cyclic light conditions does not adversely affect photoreceptor morphology (see also Ref. 12). However, in the course of these experiments, we observed a notable acceleration of the response shut off in rods overexpressing GRK1 (Fig. 2C). A more systematic examination revealed a significant \((P < 0.001)\) shortening of the response duration \((\tau_{rot})\) from 370 ms to 259 ms as a result of the GRK1 overexpression (Table 1). The time to peak was also significantly \((P = 0.01)\) reduced in the Grk1\(+\) strain at 166 ms compared with 184 ms in WT rods (Table 1).

The Overexpression of Rhodopsin Kinase Has No Effect on Light Sensitivity in Rods

Even though transretinal recordings provide a reliable and convenient measure of photoreceptor sensitivity,\(^{20}\) residual response components from second-order neurons and Müller cells make a careful analysis of response kinetics difficult. Thus, to evaluate the effect of varying GRK1 levels on sensitivity and response kinetics, we performed suction electrode recordings from individual mouse rods expressing different levels of the GRK. The response waveforms from WT rods (Fig. 3A) were comparable with those from rods with GRK1 deficit (Fig. 3B) or excess (Fig. 3C). Both Grk1\(+\) and Grk1\(+/-\) rods produced saturating responses with amplitudes comparable with those in WT rods (Table 2). Thus, altering the kinase expression level did not affect the number of open cGMP-gated channels in darkness or the basal activity of the phototransduction cascade consistent with our transretinal recordings (Fig. 2) and with the absence of degeneration in the transgenic retinas.\(^{12}\) Comparison of the half-saturating flash intensity \((I_0)\), derived from the intensity-response function for each rod, showed no overexpression effect on the light sensitivity (Table 2). Consistent with this, the estimated amplitudes of their single-photon responses remained unaffected in the GRK1-overexpressing rods (Fig. 3D). In contrast, the Grk1\(+/-\) rods showed a slight but statistically significant \((P < 0.0001)\) decrease in \(I_0\) (Table 1) indicating an increase in sensitivity. The corresponding fractional single photon response amplitude \((a/R_{max})\) was also increased in Grk1\(+/-\) compared with WT rods (Fig. 3D). This result is consistent with the observations of Doan et al.\(^{11}\) and of Chen et al.\(^{19}\)

The Expression Level of Rhodopsin Kinase Modulates the Response Shut Off Kinetics for Dim Flashes but Not for Saturating Flashes

It has been previously suggested that the overexpression of GRK1 in mouse rods does not affect the kinetics of their light responses.\(^{9}\) However, examination of the transretinal (Fig. 2C) and single-photon (Fig. 3D) responses of our Grk1\(+\) rods revealed a surprising acceleration of response termination compared with WT rods. The effect of rhodopsin kinase expression level on the kinetics of response termination of mouse rods could be better appreciated from their normalized dim flash responses (Fig. 4A). The threefold reduction in rhodopsin kinase on the deletion of one copy of Grk1 slowed down the response shutoff, as previously shown.\(^{11}\) On the other hand, contrary to the report by Krispel et al.,\(^{7}\) the threefold overexpression of rhodopsin kinase in the Grk1\(+\) mice resulted in notable acceleration of the response shut off. Accordingly, the integration time was also significantly higher in Grk1\(+/-\) rods \((P < 0.005)\) and lower in Grk1\(+\) rods \((P < 0.0001)\) compared with WT controls (Table 2). The recovery time constant \((\tau_{rec})\), believed to reflect the rate-limiting step of the rod phototransduction inactivation, was also affected by the expression level of rhodopsin kinase. It was significantly increased in Grk1\(+/-\) rods \((P < 0.0005)\) and decreased in Grk1\(+\) rods \((P < 0.05)\) (Table 2, and Fig. 4B). To ensure that our measurements reflect the recovery time constant and are not affected by reactions early on in the response shut off, we used only the second half of the response shut off for the single exponential fit determining \(\tau_{rec}\) (Fig 4A, inset). Together, these results demonstrate that, unexpectedly, the dim flash response shut off accelerates with increasing expression of GRK1 leading to a reduction in the recovery time constant. Grk1\(+\) rods expressing rhodopsin kinase at twice the WT level also showed a trend of accelerated response shut off, though the difference with WT rods did not reach statistical significance (Table 1, and Fig. 4B).

Thus, the effect of GRK1 overexpression appears to be dose-dependent so that a clear physiological phenotype becomes detectable only when GRK levels become sufficiently higher than in WT rods.

In addition to \(\tau_{rec}\), we measured a second kinetic parameter, the dominant time constant \((\tau_D)\) used to describe the inactivation of the phototransduction cascade. Whereas \(\tau_{rec}\) is derived from dim flashes responses not exceeding 30% of the

| Table 1. Parameters of Transretinal ERG Recordings |
|-----------------|-----------------|
| **WT Retina**   | **Grk1+ Retina** |
| Sensitivity, photons \(\mu m^{-2}\) | 70 ± 7 (6) | 72 ± 8 (6) |
| \(R_{max}\) \(\mu V\) | 97 ± 13 (8) | 115 ± 13 (6) |
| Time to peak, ms | 184 ± 2 (6) | 166 ± 6 (6)* |
| Integration time, ms | 370 ± 18 (8) | 259 ± 13 (6)* |

Values given as mean ± SEM \((n)\).

* Significantly different at 5% level.
† Significantly different at 1% level.
TABLE 2. Response Parameter of Single-Cell Recordings

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Grk1+/−</th>
<th>Grk1+</th>
<th>Grk1+b</th>
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<tbody>
<tr>
<td>Dark current, pA</td>
<td>14.9 ± 0.6 (36)</td>
<td>13.7 ± 0.9 (16)</td>
<td>13.8 ± 0.6 (21)</td>
<td>14.9 ± 0.6 (24)</td>
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<td>Sensitivity Io, photons μm−2</td>
<td>27.5 ± 2.0 (36)*</td>
<td>17.0 ± 1.2 (15†)</td>
<td>27.3 ± 2.0 (21)*</td>
<td>31.4 ± 2.8 (24)‡</td>
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<td>Time to peak, ms</td>
<td>176 ± 4 (36)</td>
<td>187 ± 5 (15)</td>
<td>180 ± 6 (21)</td>
<td>175 ± 5 (24)</td>
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<tr>
<td>Integration time, ms</td>
<td>356 ± 21 (36)‡</td>
<td>570 ± 50 (15)§</td>
<td>254 ± 15 (21)§</td>
<td>338 ± 22 (24)‡</td>
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<tr>
<td>Recovery time constant, τrec, ms</td>
<td>223 ± 9 (36)‡</td>
<td>343 ± 38 (15)§</td>
<td>182 ± 16 (21)†‡</td>
<td>211 ± 16 (24)‡</td>
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<tr>
<td>Dominant time constant of recovery, τp, ms</td>
<td>271 ± 13 (26)</td>
<td>271 ± 28 (11)</td>
<td>274 ± 17 (14)</td>
<td>251 ± 11 (16)</td>
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</tbody>
</table>

Values given as mean ± SEM (n). Recovery time constant (τrec) was determined by fitting a single exponential function to the second half of the shut off of the dim flash response. Dominant recovery time constant of recovery (τp) was determined by the slope of Pepperberg plot. Statistical analysis was carried out by one-way ANOVA with the post hoc Tukey Honestly Significant Difference test.

§ Significantly different from WT at 1% level.
† Significantly different from WT at 5% level.
‡ Significantly different from Grk1+/− at 1% level.
* Significantly different from Grk1+/− at 5% level.

The Expression Level of Rhodopsin Kinase Modulates the Rod Adaptation to Background Light

The activity of rhodopsin kinase in rods is modulated by the calcium-binding protein recoverin.21,22 We investigated how the recoverin to rhodopsin kinase ratio modulates rhodopsin shut off and background adaptation. The effect of rhodopsin shut off during background adaptation can be studied using the time of saturation, Tsat, of bright flash responses. As a saturating flash always reduces intracellular calcium to its lowest level, a decrease in Tsat in background light would reflect a reduction in the light-evoked phosphodiesterase activity due to shortened lifetime of photoactivated rhodopsin.23 Thus, we determined how background light affects the time of saturation of bright flash responses in Grk1+/−, WT, and Grk1+ mouse rods (Fig. 5).

As expected,22 in WT rods, exposure to background light of increasing intensity resulted in progressively faster response shut off with a corresponding decline in Tsat consistent with a stronger PDE adaptation and kinase modulation by recoverin (Figs. 5C and 5D). Conversely, a threefold overexpression of rhodopsin kinase in Grk1+ rods, or in effect reducing the recoverin:GRK1 ratio by 3 folds led to a steeper Tsat decline consistent with a stronger PDE adaptation and kinase modulation by recoverin (Figs. 5C and 5D). Together, these results indicate that the ratio of rhodopsin kinase to its modulator recoverin is critical for the proper adaptation of rods and the acceleration of their response shutoff in background light.

DISCUSSION

We investigated whether the expression level of rhodopsin kinase modulates the phototransduction amplification and the kinetics of the light response in mouse rods. This is one of the very few studies known to the authors in which the impact of varying expression levels of individual signaling proteins have been determined. Previous studies have focused on rhodopsin,24,25 transducin,26 RGS9,27 and arrestin.11,27,28

In our study, we systematically varied the expression level of GRK1 at 30% (Grk1+/−), 100% (WT), 200%...
GRK1 Expression and Rod Sensitivity

We found that sensitivity was only subtly impacted by the expression level of GRK1. A threefold overexpression of Grk1+/+ in rods had no significant effect on their sensitivity or single photon response amplitude (Tables 1 and 2, and Fig. 3D). This lack of overexpression effect on rod sensitivity indicates that WT rods express a sufficiently high level of GRK1 to allow efficient inactivation of rhodopsin under dim light conditions. In contrast, a reduction of rhodopsin kinase expression in Grk1+-/− rods to 30% of its WT level did increase the sensitivity of mouse rods (Tables 1 and 2). Accordingly, their single photon responses were also larger in amplitude (Fig. 3D). Thus, reducing rhodopsin kinase expression threefold hindered the inactivation of rhodopsin enough to subtly but significantly affect the amplitude of the dim-flash response. A similar result was recently reported by Doan et al.,11 as well as by Chen et al.,29 who found only a small increase in rod sensitivity even when the expression of rhodopsin kinase was reduced to 15% of the WT level. Notably, even the complete deletion of rhodopsin kinase in the Grk1−/− rods results in only a two-fold increase in the dim flash response amplitude.19 Whole-retina recordings have also demonstrated the lack of significant increase of sensitivity in mouse rods lacking GRK1.30 Thus, it appears that the expression level of rhodopsin kinase has only a minor effect on the amplitude of the response. As the peak of the response reflects the balance of cGMP hydrolysis by PDE and its synthesis by guanylyl cyclase, its amplitude is controlled by the strong activation of cGMP synthesis induced by the reduction in intracellular calcium. Induced during the light response, this negative feedback produces a substantially larger 5- to 6-fold change in the response amplitude and a more dramatic change in its shut off kinetics than the deletion of rhodopsin kinase.31,32

GRK1 Expression and the Shut Off Kinetics of Rod Dim Flash Responses

The recovery of the dark current after light stimulation requires inactivation of all phototransduction intermediates, including photoactivated rhodopsin, R*, and the active form of transducin bound to PDE, Ta/PDE. The relative rates of inactivation of these two intermediates and which one represents the rate-limiting step for response shut off has been a subject of debate. One recent study examined this question using mouse rods overexpressing either RGs9 or GRK1 to speed up the inactivation of Ta/PDE or R*, respectively.9 This study clearly demonstrated that acceleration of Ta/PDE inactivation produces a dramatic reduction in both Trec and Tη, and concluded that the shut off of the flash response in both dim light (as measured by Trec) and in bright light (as measured by Tη) is rate-limited by the inactivation of the Ta/PDE complex. Notably, the authors also stated that overexpression of GRK1 had no effect on the rod dim flash or saturated response shut off. The conclusion drawn from these results was that the lifetime of R* is significantly shorter than that of Ta/PDE so that acceleration of R* shut off would not affect the overall response kinetics. Indeed, a more recent study10 suggested that the time constant of R* inactivation is only ~40 ms compared with ~250 ms for the inactivation of Ta/PDE.

However, in contrast to the results by Krispel et al.,9 our results clearly demonstrate that the expression level of rhodopsin kinase does modulate the shut off kinetics of dim flash response. We find that reducing the level of rhodopsin kinase threefold in Grk1+/− rods results in a substantial slowing of the dim flash response termination and a significant increase in the recovery time constant. More importantly, increasing kinase expression level threefold in Grk1+/+ rods accelerates their dim flash response termination and decreases significantly the recovery time constant (Table 2, and Figs. 4A and 4B). The disparity could originate in our use of the full-length BAC rhodopsin kinase promoter which produced uniform GRK1 expression across photoreceptors (Fig. 1) as opposed to the opsin promoter used by Krispel et al.,9 which may have a less uniform activity profile.35,34 Alternatively, the discrepancy between our results could be due to species differences as we used mouse GRK1 in our study in contrast to Krispel et al. who used bovine GRK1.

Though it is possible that rhodopsin kinase somehow directly modulates the inactivation of Ta/PDE, the most likely interpretation of our results is that the overexpression of rhodopsin kinase speeds up the inactivation of rhodopsin by phosphorylation to result in faster response shut off. Thus, while rhodopsin inactivation is clearly not the rate-limiting reaction for phototransduction inactivation, its rate does appear to be close enough to the one of Ta/PDE inactivation to affect the overall response shut off kinetics. If Ta/PDE decay dominates the response shut off after R* inactivation, then increasing or decreasing the lifetime of R* by varying the

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933460/)
expression level of rhodopsin kinase might shift the shut off to the right or to the left, respectively, thus affecting the overall kinetics of the response.

These results are consistent with recent findings by Doan et al., who demonstrated that modulation of the rate of rhodopsin inactivation by arrestin can also affect the response shut off and estimated the time constant for R* inactivation at ~150 ms. Notably, our flash responses are comparable to those reported by Gross and Burns, both recorded in Locke's solution, and are substantially faster and smaller than those reported by Doan et al. who used Ames perfusion solution.

Finally, our results clearly demonstrate that whereas \( \tau_{\text{rec}} \) is modulated by the expression level of rhodopsin kinase, \( \tau_0 \) is not. Thus, whereas in WT rods these two parameters have similar values, the two kinetic parameters can be uncoupled and modulated independently of each other in mouse rods so that the expression level of GRK1 modulates \( \tau_{\text{rec}} \) but has no effect on \( \tau_0 \). As recently argued by Gross and Burns, the measurement of \( \tau_{\text{rec}} \) might not allow sufficient time for R* to be turned off. In contrast, as the measurement of \( \tau_0 \) is done a relatively long time after the test flash, its value would reflect only the slowest inactivation step of the phototransduction cascade. Thus, it appears that the lifetime of photoactivated rhodopsin is long enough to modulate \( \tau_{\text{rec}} \) but not \( \tau_0 \).

**GRK Expression and Background Adaptation**

The activity of rhodopsin kinase in rods is modulated by recoverin. In darkness, when intracellular calcium is high, recoverin inhibits the phosphorylation of photoactivated rhodopsin by rhodopsin kinase, thus prolonging its activity. The decline in calcium when the photoreceptor is exposed to light would relieve rhodopsin kinase of its inhibition by recoverin and speed up rhodopsin inactivation. This represents one possible mechanism by which the modulation of the rod phototransduction cascade could occur during background light adaptation. The recoverin content in mouse rods is believed to exceed that of rhodopsin kinase by an order of magnitude. Altering this ratio by either increasing or decreasing the level of rhodopsin kinase in rods could potentially reduce or enhance the effectiveness with which recoverin inhibits its activity. Indeed, our results demonstrate that reducing the expression level of GRK1 threefold enhances the modulation of \( \tau_0 \) in background light (Fig. 5C). Conversely, the threefold overexpression of GRK1 essentially abolishes the modulation of \( \tau_0 \) by background light (Fig. 5C). Interestingly, this lack of PDE adaptation in rhodopsin kinase overexpressing rods is reminiscent of the case in recoverin null mice. Thus, weakening the calcium modulation of rhodopsin kinase by either deleting recoverin or by increasing the ratio of rhodopsin kinase to recoverin in Grk1+ rods has a similar effect on PDE adaptation. Together, our results indicate that the inhibition of rhodopsin inactivation by recoverin modulates the lifetime of R* and that this modulation is important for the ability of mouse rods to adapt to background light. As recent studies have shown the importance of visual pigment phosphorylation in modulating the pathogenesis of retinal disease in animal models, control of visual pigment phosphorylation could also be an important parameter in how photoreceptors respond and adapt to environments of varying light intensity. Finally, the GRK1 transgenic mice will be a valuable resource for future studies of the role of GRK in response shut off in cones.

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**References**


