Transduction noise induced by 4-hydroxy retinals in rod photoreceptors

D. Wesley Corson,*‡ M. Carter Cornwall,§ E. F. MacNichol,§ Venkatchalam Mani,‡ and Rosalie K. Crouch‡

*Department of Pathology and Laboratory Medicine and ‡Department of Ophthalmology, Medical University of South Carolina, Charleston, South Carolina 29425; and §Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT New visual pigments were formed with 4-hydroxy retinals in isolated vertebrate rod photoreceptors by exposing bleached rods from the tiger salamander, *Ambystoma tigrinum*, to lipid vesicles containing the analogues. Formation of physiologically active pigment was demonstrated by the restoration of sensitivity and by a shift of ~50 nm in the peak of both the visual pigment absorptance spectrum and rod spectral sensitivity spectrum from ~520 to ~470 nm for 11-*cis* 4-hydroxy retinal. Membrane current recordings from the inner segments of isolated rods revealed excess fluctuations in membrane current after formation of the new pigment in bleached cells or after exposure of unbleached cells to flashes in the presence of the analogue. The excess current fluctuations are similar to the fluctuations elicited by steady light producing a few discrete responses per second, a rate ~100 times greater than the normal rate of spontaneous events in darkness. These results suggest that analogues of retinal can produce alterations in the frequency of production of discrete responses in darkness in rod photoreceptors.

INTRODUCTION

Absorption of a photon elicits a discrete electrical response in rod photoreceptors after isomerization of the 11-*cis* retinal chromophore of one rhodopsin molecule (1–5). Little is known about the role of rhodopsin in controlling the quantum efficiency of discrete response production, the shape of individual responses, or the rate of occurrence of spontaneous quantal responses in darkness. Previously, only two mechanisms were known to elevate the rate of occurrence of discrete responses in rods in darkness. These mechanisms are (*a*) thermal isomerization of rhodopsin produced by elevated temperature (6–8) and (*b*) latent back-reactions among photoproducts in the bleaching cascade which can occur for many minutes after the exposure of cells to bright light (9–13). Seeking biochemical probes for the control of discrete wave production at rhodopsin, we have begun to explore the role of retinal in the generation of spontaneous and light-evoked responses (14, 15). We began with 4-hydroxy analogues of retinal because they are known to form thermally stable and spectrally distinct visual pigments with vertebrate opsin in vitro (16, 17), and physiologically functional pigments in invertebrates where 3-hydroxy retinal and its 4-hydroxy relative have been found to occur naturally in the visual pigments of insects (18) and a mollusc (19). These recently discovered retinal analogues are the third and fourth known visual pigment chromophores in the animal kingdom, and the 4-hydroxy analogue has been found to participate along with 11-*cis* retinal and 11-*cis* 3,4-dehydro retinal in a unique trichromatic visual system in the squid *Watasenia scintillans* (19).

We have found (*a*) that 4-hydroxy analogues of retinal form functional and spectrally distinct visual pigments in isolated vertebrate rod photoreceptors, and (*b*) that in the darkness after bright lights, the 9- and 11-*cis* isomers of 4-hydroxy retinal produce noise (fluctuations in membrane current) in rods that is not seen under the same conditions with 9- or 11-*cis* retinal or with all-*trans* 4-hydroxy retinal. Power spectral density analysis of this analogue-induced noise shows that it contains frequency components characteristic of the single photon responses underlying photon shot noise. These results suggest that the rate of production of discrete responses in darkness can be elevated in rod photoreceptors with biochemical probes in addition to heat and bright light.

METHODS

The 9-*cis* and all-*trans* retinals were obtained from Sigma Chemical Co. (St. Louis, MO). 11-*cis* retinal was the generous gift of Hoffmann-La Roche (Nutley, NJ). The 9-*cis*, 11-*cis* and all-*trans* isomers of 4-hydroxy retinal were synthesized according to the method of Renk and Crouch (20). All retinals were examined for purity and isomeric specificity by absorption spectrometry and high-pressure liquid chromatography (HPLC) with the criterion for purity being >99%. If necessary, the retinals were rechromatographed by HPLC (u-porasil, hex-

Address correspondence to D. Wesley Corson, Ph.D., Department of Pathology and Laboratory Medicine, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.

0006-3495/90/01/109/07 $2.00

ane/ethyl acetate solvents) before use. Lipid vesicles containing retinal were formed by sonication of a mixture of 1 mg of retinal, 25 mg of l-α-phosphatidylcholine (Sigma Chemical Co.) and 10 ml of *Ambystoma* Ringer (21, 22).

Changes in membrane currents evoked by light and by 4-hydroxy retinals were recorded from the inner segments of mechanically isolated rods from the tiger salamander, *Ambystoma tigrinum*, (Waterdog Farms, Tulsa, OK) by means of a suction pipette connected to a conventional current-to-voltage converter (23, 24). Visual pigments incorporating different chromophores were produced by exposing bleached outer segments of cells to lipid vesicles containing synthetic retinals. 11-*cis* retinal applied in this way has been reported to restore sensitivity in isolated bleached rods and cones (25). The animals were purchased in larval form in which the chromophore is essentially all 11-*cis* 3,4-dehydro retinal (26). They were maintained in a chilled aquarium to prevent metamorphosis to adults in which the chromophore is a mixture of 11-*cis* retinal and 11-*cis* 3,4-dehydro retinal, a condition which would complicate the measurement of spectral shifts (26).

Power spectral density measurements closely followed the methods described by Baylor et al. (1). Briefly, records for noise analysis were collected on an FM instrumentation recorder (Racal Store 4DS, Rockville, MD), filtered at 20 Hz with an eight-pole Bessel Filter (model 902LPF1, Frequency Devices, Haverhill, MA), and digitized on a laboratory computer. Power spectral density measurements were made according to the methods of Baylor et al. (1) with the aid of ASYST software (ASYST Technologies, Inc., Rochester, NY). The measurement procedures were verified by analysis of a synthetic data set of simulated photon shot noise from rods constructed from the parameters originally measured by Baylor et al. (1).

In the analysis used here for recordings from rod inner segments, we follow the model developed by Baylor et al. (23) for current recordings for rod outer segments. In essence this model assumes that the absorption of photons can be described as a simple exponential Poisson statistical process and that the unit response to a single photon can be modeled by a fourth order Poisson kinetic model given here in Eq. 1 which is equivalent to Eq. 3 of Baylor et al. (23). In Eq. 1, $r^*(t)$ is the

$$r^*(t) = i\,S\,T^{n-1}\exp\left[(n-1)(1-T)\right] \qquad (1)$$

response of the cell at time t, after a flash for a given flash intensity i and flash sensitivity S. The number of stages in the model is given by n and the normalized time is T ($T = t/t_{peak}$). Under these assumptions, we estimate the amplitude (a) of the average discrete response from the ratio of the variance (σ^2) of the noise to the mean (μ) and a shape factor ($s = 1.43$ for Eq. 1) as given in Eq. 2, a statement of Campbell's theorem (27):

$$a = s\,\sigma^2/\mu. \qquad (2)$$

The rate of occurrence (ν) of discrete responses is estimated from the height of the zero frequency asymptote ($S[0]$) of the power spectrum and the amplitude (a) and time to peak (t) of the quantal response via Eq. 3, which is equivalent to Eq. 20 of Baylor et al. (1):

$$\nu = S(0)/4.43\,a^2\,t^2. \qquad (3)$$

The optical bench in the physiological recording apparatus contains a tungsten/halogen lamp operated at 6.6 A, which delivered 4.7×10^7 photons/μm^2s to the preparation at full intensity as measured with a PIN photodiode radiometer (model 111, United Detector Technology, Santa Monica, CA). To estimate the expected rate of quantal responses in steady light and the fraction of pigment bleached, we take the effective collecting area of an *Ambystoma* rod to be 18 μm^2 (1, 28) and the total number of pigment molecules to be 3×10^9 (26).

Microspectrophotometric measurements of analogue pigment were made with a single beam, photon-counting microspectrophotometer (PMSP) (29, 30). Briefly, a suspension of isolated rod outer segments and intact cells was placed in a test tube and completely bleached with a microscope lamp and cut-off filter of >560 nm for 5 min. The suspension was then mixed with vesicles containing analogues and placed in a darkened container for ~1 h. At that time, 20 mM hydroxyl amine (Sigma Chemical Co.) was added and the cells were pipetted into a 70-μm deep chamber between two cover slips and placed in the PMSP for measurements. An optical density spectrum was recorded and used to compute the absorptance of the pigment as described previously (31).

RESULTS

The 9- and 11-*cis* isomers of 4-hydroxy retinal form visual pigments that are physiologically active and spectrally distinct from the native pigment. After a bleach of >90% of the native pigment (complete bleach) but in the absence of added retinal, the amplitude of flash responses (responsivity) partially recovered to about two-thirds of the prebleach amplitude (66 ± 20%; mean ± SD; $n = 5$ cells) over the course of 30–50 min in darkness. The reciprocal of the photon flux required to elicit a criterion response (sensitivity) also recovered partially but remained depressed by ~3 log units below the dark-adapted level in the absence of pigment regeneration in these isolated cells as shown previously (24). When vesicles containing 11-*cis* 4-hydroxy retinal were added, a new and physiologically active visual pigment was formed as shown in Fig. 1. This is evidenced by the 48-nm shift in the spectral sensitivity peak from 519 to 471 nm and by restoration of responsivity to near the prebleach level (Fig. 1, *inset*, fourth trace from top). Whereas the recovery of responsivity was substantially complete in all five cells, recovery of sensitivity from the 3-log unit desensitization was variable from cell to cell (1.6–3.2 log units) but was comparable with that found with 11-*cis* retinal. The spectral sensitivity maximum is in good agreement with the absorptance maximum of 472 nm measured in two tiger salamander outer segments by microspectrophotometry (Fig. 1, *solid dots*) and close to the 470-nm peak of the in vitro bovine pigment reported by Renk and Crouch (17). Application of 9-*cis* 4-hydroxy retinal in three bleached cells produced physiological effects similar to 11-*cis* 4-hydroxy retinal but the spectral sensitivity and absorptance maxima were further shifted to 461 and 468 nm as measured in two and three cells, respectively (results not shown). The formation of new pigment was essentially complete within 1 h.

Although there was substantial recovery after addition of the 4-hydroxy retinal analogue, the cells did not return to a normal state but instead exhibited excess fluctuations of membrane current which persisted in darkness (Fig. 1, *inset*, fifth trace from top). This noise resembled the

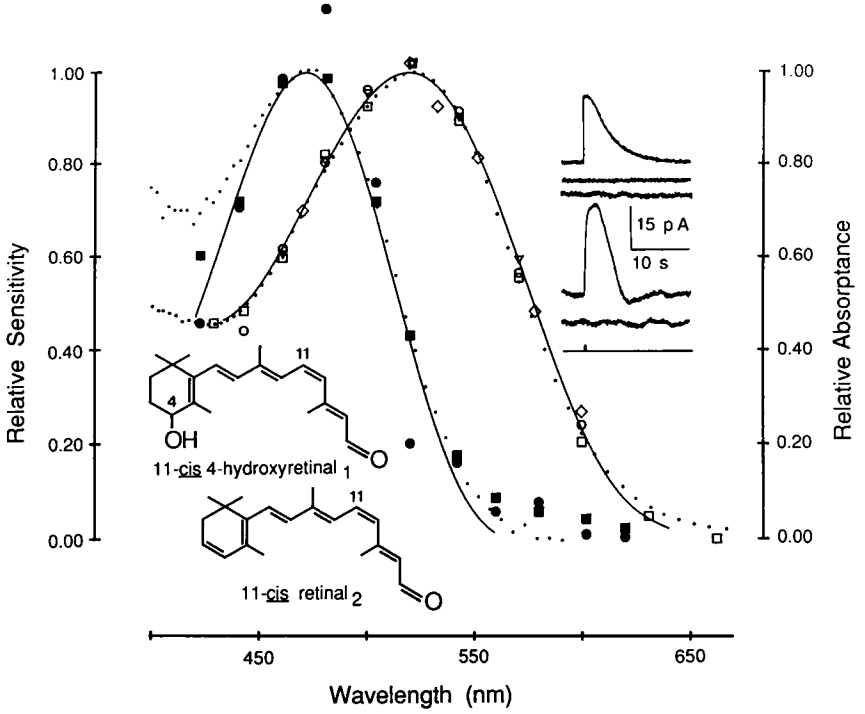

FIGURE 1 Blue-shifted absorptance and spectral sensitivity of an 11-*cis* 4-hydroxy retinal visual pigment analogue (*left curve, solid symbols*). The sensitivities of two isolated cells were measured after regeneration of their visual pigments with 11-*cis* 4-hydroxy retinal (*solid symbols*). A spectral sensitivity peak at 471 nm was measured by fitting a wavelength-compensated polynomial template whose shape was derived from microspectrophotometric absorbance measurements of the vitamin A_1 pigment in *Bufo* rods (smooth curve, Jones, G., personal communication). The spectral sensitivity data and template match reasonably closely to the average absorptance spectrum (*solid dots*) of the 11-*cis* 4-hydroxy analogue pigment measured in two cells. For comparison, previously published spectral sensitivity measurements of the native vitamin A_2 based pigment (*open symbols*, right curve, reference 23) are best fit with a peak at 519 nm by an absorbance template (*solid curve*) derived from the A_2 pigment from *Necturus* rods. The spectral sensitivity measurements of the native pigment are in good agreement with the absorptance measurements of the native pigment in situ (*solid dots*, reference 23). Inset at upper right shows a near-maximal flash response (*top trace*), a recording of membrane current in darkness (second trace from top) and photon shot noise induced by dim steady light (3 Rh*/s, third trace from top) taken before bleaching. A response evoked by a flash of the same intensity and a section of dark record after resensitization with 11-*cis* 4-hydroxy retinal are shown in the fourth and fifth traces from the top, respectively. The sixth trace from the top in the inset shows the occurrence of the 200-ms test flash.

photon shot noise evoked by dim steady light (Fig. 1, *inset*, third trace from top). Five cells completely bleached and subsequently exposed to *cis* 4-hydroxy retinals all produced this additional component of noise after resensitization in darkness (11-*cis* 4-hydroxy retinal, $n = 2$; 9-*cis* 4-hydroxy retinal, $n = 3$). This increase in noise could be measured as a 3.4 ± 1.2 (mean ± SD, $n = 5$)–fold increase in the variance of the membrane current over the control value of 0.079 ± 0.037 pA^2 obtained in darkness at the beginning of the experiment. This control level of the variance of the baseline noise is within the range observed by Baylor et al. for recordings from toad outer segments (1). Visual inspection of records obtained with control compounds on seven completely bleached cells did not reveal similar noise (11-*cis* retinal, $n = 2$; 9-*cis* retinal, $n = 3$; all-trans retinal, $n = 1$ and all-*trans* 4-hydroxy retinal, $n = 1$). The all-*trans* retinals did not restore sensitivity.

To determine if the long-lasting component of membrane noise elicited by the synthetic retinals required both prebleaching and the 4-hydroxyl group, we exposed unbleached rods (< 0.1% bleach by test lights) to solutions containing test substances. In darkness, these cells did not exhibit long-lasting current fluctuations comparable with those observed in bleached cells. However, the 4-hydroxy retinal analogue did produce a transient elevation in current fluctuations after test flashes (5,400 Rh*) as illustrated in Fig. 2. Under control conditions, a cell in saline solution (Fig 2., Column 1) produced typical responses to test flashes (*Flashes*) and a level of membrane current noise typical of dark-adapted cells both immediately after the flashes (*Dark 1*) and many minutes later (*Dark 2*). A dim steady light (3 Rh*/s, *Dim*) elicited membrane current fluctuations typical of the shot noise produced by the summation of single photon responses (1). A bright light pulse of 100-s duration

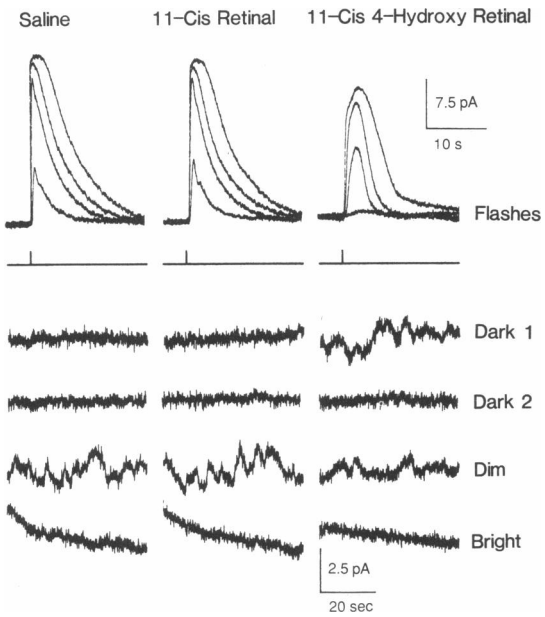

FIGURE 2 Induction of noise by 11-*cis* 4-hydroxy retinal. (*Column 1*) Saline control before exposure to vesicles. (*Flashes*) Responses to flashes (520 nm, 200 ms) at four intensities (170, 540, 1,700, 5,400 Rh*/s) at the time indicated by the light monitor (LM). (*Dark 1*) membrane current noise in darkness starting 1 min after a flash (variance, s^2 = 0.042 pA^2). (*Dark 2*) membrane current noise in darkness 25 min after flash (s^2 = 0.050 pA^2), (*Dim*) Photon shot noise elicited by dim steady light (3 Rh*/s, s^2 = 0.209 pA^2). Bright, A bright steady light (8,500 Rh*/s, 100 s) reduces membranes current fluctuations by saturating the receptor response. (*Column 2*) 11-*cis* retinal vesicle control. Replication of the conditions in the saline control column after application (and removal) of vesicles containing 11-*cis* retinal, a control analogue which is also the native chromophore of the adult salamander but not the larval form. The flash responses and membrane current noise were not noticeably different from the unexposed saline control (s^2 = 0.040, 0.039, and 0.340 pA^2 for *Dark1*, *Dark2* and *Dim*, respectively). (*Column 3*) 11-*cis* 4-hydroxy retinal. Subsequent application of vesicles containing 11-*cis* 4-hydroxy retinal and exposure to test flashes produced attenuated flash responses (*Flashes*) and a noisy current record in darkness after the flashes (s^2 = 0.2491 pA^2, *Dark 1*). After many minutes in darkness, the noise returned to control levels (s^2 = 0.044 pA^2, *Dark 2*). A dim steady light (3 Rh*/s, Dim) did not change the character of the membrane noise (s^2 = 0.095 pA^2), but a bright steady light (*Bright*, 8,500 Rh*/s, 100 s) suppressed the analogue-induced noise.

(8,500 Rh*/s, *Bright*) nearly saturated the light-evoked change in membrane current and thereby suppressed the current fluctuations seen at lower intensities. No changes in any of these responses were seen if cells were exposed to vesicles containing 11-*cis* retinal (Column 2). However, if the same cells were subsequently exposed to 11-*cis* 4-hydroxy retinal (Column 3), identical flashes now evoked smaller responses and substantial membrane current fluctuations lasting several minutes in the darkness immediately after the flashes (Column 3, *Dark 1*). This excess membrane current noise diminished slowly to control levels over the course of many minutes (Column 3, *Dark 2*) but could be reelicited by subsequent flashes. Except for its decay in darkness, it is otherwise similar to that noise which persists after exposure of bleached cells to 9- and 11-*cis* 4-hydroxy retinals. Noise elicited by *cis* 4-hydroxy retinals in both of these conditions appears qualitatively similar to photon shot noise and like photon shot noise (1) can be suppressed by bright light (Column 3, *Bright*) as would be expected if both photon shot noise and analogue-induced noise share all or part of a common biochemical pathway. The analogue-induced noise shown in Fig. 2 is also similar to photon shot noise in that the responses to flashes are attenuated in the presence of the analogue-induced noise (Column 3, *Flashes*) as they would be in the presence of dim steady background light (23). Similar excess membrane current fluctuations were observed in six additional unbleached cells exposed to 11-*cis* 4-hydroxy retinal and three unbleached cells exposed to 9-*cis* 4-hydroxy retinal. Control observations on seven unbleached cells, with 11-*cis* retinal (n = 5) and all-*trans* 4-hydroxy retinal (n = 2) did not show similar current fluctuations. The excess noise induced by analogues under the conditions shown in Fig. 2 differed from the light-induced dark noise reported by Lamb (9) in that it appeared only in the presence of 9- or 11-*cis* 4-hydroxy retinal and did not appear after the flashes in our control measurements which bleached 1,000-fold less pigment than the flashes used by Lamb.

We measured the power spectral density of the analogue-induced noise by previously described methods (1) to determine whether it contained the frequency components characteristic of the discrete responses underlying photon shot noise. Power spectral density was measured in cells under three different conditions: (*a*) unbleached cells exposed to dim light, (*b*) bleached cells resensitized with 9- or 11-*cis* 4-hydroxy retinals, and (*c*) cells with a nearly complete complement of unbleached native pigment which had recently been exposed to 100-s-long bright flashes in the presence of 4-hydroxy retinals. Flash durations of 100 s were chosen for the noise measurements under condition *c* because they produced levels of excess analogue-induced current fluctuations comparable to light-evoked fluctuations (3 Rh*/s) for longer than the 3.5 min required for power spectral measurements. The example in Fig. 3 shows a comparison of power spectral densities of membrane noise from a cell exposed to dim light (condition *a*, *open circles*) and from the same cell after exposure to 11-*cis* 4-hydroxy retinal and long flashes (2.7×10^4 Rh*/s, 100 s, condition *c*, *open squares*). These data are reasonably fitted by the spectral curve predicted by the kinetics of the average dim flash response fitted by a Poisson model with four first-order stages of delay (*solid line*, Eq. 1, reference 23). Noise

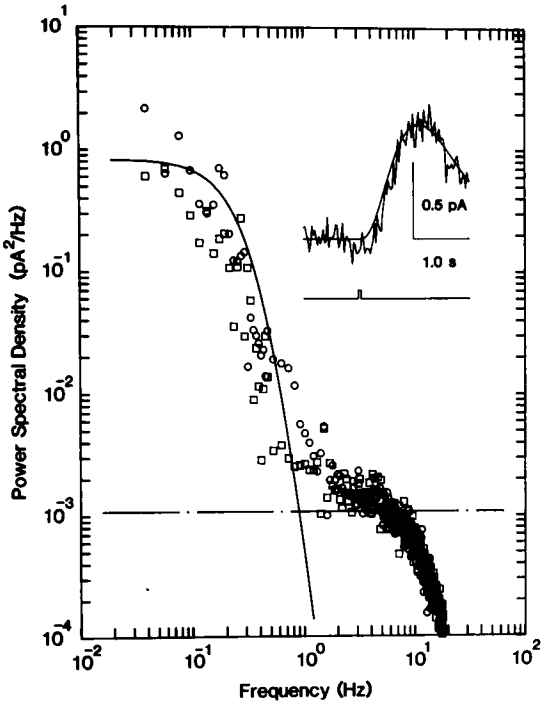

FIGURE 3 Power spectral density of noise induced by 4-hydroxy retinal (*open squares*) and dim steady light (*open circles*). Four records of 51.2 s duration were sampled, processed, averaged, and fitted with a kinetic model as described by Baylor et al.(1). The smooth curve fitted through the data points is the power spectrum of the product of four Lorentzians predicted by a fourth order Poisson activation model (Eq. 1) with a time to peak of 1.1 s obtained by fitting the model (*smooth curve, inset*) to the average of 12 dim flash responses (*solid curve, inset*). The lower dotted curve is the level of background Johnson noise predicted for the 15 MΩ leakage resistance of the recording electrode.

spectra from a second cell in condition *c* and from two cells bleached and regenerated with 11-*cis* 4-hydroxy retinal (condition *b*) were all found to be similar to noise spectra taken from each cell in the presence of photon shot noise before bleaching (condition *a*). Based on the calibrated photon flux and the effective collecting area of the outer segments, we estimate the rate of effective photoisomerizations in the dim light conditions in Figs. 2 and 3 to be of the order of three per second (1, 28). This rate is in good agreement with a mean rate of one event per second estimated from the power spectrum (Eq. 3) and a peak amplitude of single photon responses of 0.25 pA calculated from the mean and variance of the noise via Eq. 2 (Fig. 3, references 1, 23, 32). Assuming that the 4-hydroxy analogues elicit discrete events of similar magnitude, we conclude from their power spectrum that these events occur at a rate comparable with that of the light-evoked events, a rate which is 100 times higher than typical rates of spontaneous discrete responses in untreated rods (7).

DISCUSSION

4-Hydroxy analogues of retinal form physiologically active visual pigments in isolated rod photoreceptors as shown in Fig. 1. The complete restoration of sensitivity in a number of cells indicates that these analogues could be efficiently transported to the rod disks and inserted into the majority of the available opsin despite the hydrophilic hydroxyl group on the cyclohexyl ring. Both the PMSP and spectral sensitivity measurements of Fig. 1 indicate that the pigment which forms in living cells has the same spectral properties as the in vitro pigments formed from bovine rhodopsin (17). Indeed, the spectral maximum is also close to the that of the more distantly related invertebrate visual pigments which have 3-hydroxy retinal (490 nm, references 18, 33) and 4-hydroxy retinal (470 nm, reference 19) as native chromophores.

The responses evoked from cells that were bleached and resensitized with 4-hydroxy analogue pigments appear to be similar to the responses of the dark-adapted cell before the bleach. For this to occur, both the quantum efficiency and catalytic activity of the new pigments must be close to normal. In addition, the spontaneous isomerization rates of the new pigments in bleached and resensitized cells cannot be substantially greater than that of the native pigment because the sensitivity and responsivity of the cells most highly resensitized by the 4-hydroxy analogues approached that of the cells before bleaching and were not highly desensitized as they would be in the presence of a bright light. Indeed, one might expect that the blue-shifted analogue pigments would have a higher energy of activation and would thus be even more stable than the native pigments which are already extremely stable (34, 35). However, the rate of spontaneous discrete electrical events in toad green rods has been reported to be higher than the rate in toad red rods (36), in contrast to expectations based on Arrhenius rate theory (34, 35). The noise observed after bleaching and resensitization with 4-hydroxy retinal may arise in part from a greater thermal isomerization rate in the newly formed analogue pigment, but we have not yet made measurements extensive enough to identify this component at its presently observed magnitude and separate it from other noise components induced by the analogues at lower intensities.

The noise observed when cells were resensitized with 4-hydroxy analogues or exposed to moderately bright flashes in the presence of the analogues appears similar to the photon shot noise elicited by a steady light producing a few photoisomerizations per second. Like photon shot noise, it is suppressed by bright light. The noise appears to arise specifically from the presence of the *cis* 4-hydroxy analogues as indicated by the controls with 11-*cis* retinal

and all-*trans* 4-hydroxy retinal. Furthermore, it appears to arise from the summation of discrete responses produced by activation of the rod transduction cascade as suggested by the data in Figs. 2 and 3. From Fig. 3, we estimate that the frequency of analogue-induced events in unbleached cells is of the order of a few per second after a bright 100-s flash. The power spectra of the analogue-induced noise after bleaching and resensitization indicate that the rates of events underlying this noise is most likely also of the order of a few per second.

Based on the similarities between the analogue-induced noise and photon shot noise, we conclude that the 4-hydroxy retinals activate an early stage in the cascade of phototransduction in a manner that produces discrete events. Based on these same similarities and the specific dependence on the *cis* isomers of the analogues, we suggest that the likely site of action is the visual pigment. Because we find comparable amounts of noise under the very different conditions described in Figs. 1 and 2, it is unlikely that all of the effects observed so far can be due to simple thermal instability of a newly formed analogue visual pigment. Rather, it seems likely that the analogue-induced noise observed in unbleached cells may arise either from facilitation of light-induced dark noise or from an as yet undescribed class of noise-inducing mechanisms. The analogue-induced noise observed after bleaching and resensitization may arise in part from thermal instability of the new pigment and in part from the mechanisms listed above. The present results do not yet allow us to distinguish among these possibilities. Most importantly, these results demonstrate that structural modifications of retinal may perturb the physiological mechanism of transduction in ways that will allow us to probe the activation of transduction cascades.

We thank Drs. Gregor Jones, John Lisman, Alan Fein, Louis DeFelice, and Paul O'Bryan for helpful discussions.

This work was supported by grants from the Medical University of South Carolina and the National Eye Institute of the National Institutes of Health (EY-07543, -04939, -6537, -01157).

Received for publication 4 April 1989 and in final form 21 August 1989.

REFERENCES

1. Baylor, D. A., T. D. Lamb, and K.-W. Yau. 1979. Responses of retinal rods to single photons. *J. Physiol. (Lond.).* 288:613–634.
2. Wald, G. 1968. Molecular basis of visual excitation. *Science (Wash. DC).* 162:230–239.
3. Baylor, D. A. 1987. Photoreceptor signals and vision. *Invest. Ophthalmol. & Visual Sci.* 28:34–49.
4. Chabre, M. 1985. Trigger and amplification mechanisms in visual phototransduction. *Annu. Rev. Biophys. Biophys. Chem.* 14:331–360.
5. Stryer, L. 1986. Cyclic GMP cascade of vision. *Annu. Rev. Neurosci.* 9:87–119.
6. Ashmore, J. F., and G. Falk. 1977. Dark noise in retinal bipolar cells and stability of rhodopsin in rods. *Nature (Lond.).* 270:69–71.
7. Yau, K.-W., G. Matthews, and D. A. Baylor. 1979. Thermal activation of the visual transduction mechanism in retinal rods. *Nature (Lond.).* 279:806–807.
8. Baylor, D. A., G. Matthews, and K.-W. Yau. 1980. Two components of electrical dark noise in toad rod outer segments. *J. Physiol. (Lond.).* 309:591–621.
9. Lamb, T. D. 1980. Spontaneous quantal events induced in toad rods by bleaching. *Nature (Lond.).* 287:349–351.
10. Lamb, T. D. 1981. The involvement of rod photoreceptors in dark adaptation. *Vision. Res.* 21:1773–1782.
11. Ashmore, J. F., and G. Falk. 1981. Photon-like signals following weak rhodopsin bleaches. *Nature (Lond.).* 289:489–491.
12. Lisman, J. 1985. The role of metarhodopsin in the generation spontaneous quantum bumps in ultraviolet receptors of *Limulus* median eye. *J. Gen. Physiol.* 85:171–187.
13. Lamb, T. D. 1987. Sources of noise in photoreceptor transduction. *J. Opt. Soc. Am.* A4:2295–2300.
14. Crouch, R. 1986. Studies of rhodopsin and bacteriorhodopsin using modified retinals. Yearly review. *Photochem. Photobiol.* 44:803–807.
15. Balogh-Nair, V., and K. Nakanishi. 1984. The stereochemistry of vision. *New Compr. Biochem.* 3:283–334.
16. Renk, G. E. 1986. Structural and functional studies on rhodopsin using electron spin resonance. Ph.D. thesis. Medical University of South Carolina, Charleston.
17. Renk, G., and R. K. Crouch. 1989. Analogue pigment studies of chromophore-protein interactions in metarhodopsins. *Biochemistry.* 28:907–912.
18. Vogt, K. 1983. Is the fly visual pigment a rhodopsin? *Z. Naturforsch. Sect. C Biosci.* 38:329–333.
19. Matsui, S., M. Seidou, I. Uchiyama, N. Sekiya, K, Hiraki, K. Yoshihara, and Y. Kito. 1988. 4-Hydroxyretinal, a new visual pigment in the bioluminescent squid, *Watasenia scintillans. Biochim. Biophys. Acta.* 966:370–374.
20. Renk, G. E., T. Grover, R. Crouch, B. Mao, and T. G. Ebrey. 1981. A spin-labeled retinal pigment analogue of the purple membrane. *Photochem. Photobiol.* 33:489–494.
21. Yoshikami, S., and G. N. Noll. 1979. Technique for introducing retinol analogs into the isolated retina. *Methods Enzymol.* 81:447–452.
22. Perlman, J. I., B. R. Nodes, and D. R. Pepperberg. 1982. Utilization of retinoids in the bullfrog retina. *J. Gen. Physiol.* 80:885–913.
23. Baylor, D. A., T. D. Lamb, and K.-W. Yau. 1979. The membrane current of single rod outer segments. *J. Physiol. (Lond.).* 288:589–611.
24. Cornwall, M. C., A. Fein, and E. F. MacNichol. 1983. Spatial localization of bleaching adaptation in isolated vertebrate rod photoreceptors. *Proc. Natl. Acad. Sci. USA.* 80:285–288.
25. Cornwall, M. C., G. J. Jones, and A. Fein. 1987. Recovery of sensitivity of isolated bleached rods and cones on exposure to liposomes containing 11-*cis* retinal. *Invest. Ophthalmol. & Visual Sci.* 28(Suppl.):401.

27. DeFelice, L. J. 1981. Introduction to Membrane Noise. Plenum Publishing Corp., New York.

26. Harosi, F. I. 1975. Absorption and linear dichroism of some amphibian photoreceptors. *J. Gen. Physiol.* 66:357–382.

28. Matthews, G. 1983. Physiological characteristics of single green rod photoreceptors from toad retina. *J. Physiol. (Lond.).* 342:347–359.

29. MacNichol, E. F., Jr. 1978. A photon counting microspectrophotometer for the study of single vertebrate photoreceptor cells. *In* Frontiers in Visual Science. J. S. Cool, and E. L. Smith, editors. Springer-Verlag, New York. 194–208.

30. Levine, J. S., and E. F. MacNichol, Jr. 1985. Microspectrophotometry of primate photoreceptors: art, artifact and analysis. *In* The Visual System. A. Fein and J. Levine, editors. Alan R. Liss, Inc., New York. 73–88

31. Cornwall, M. C., E. F. MacNichol, and A. Fein. 1984. Absorptance and spectral sensitivity measurements of rod photoreceptors of the tiger salamander, *Ambystoma tigrinum. Vision Res.* 24:1651–1659.

32. Katz, B., and R. Miledi. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol. (Lond.).* 224:665–699.

33. Ostroy, S. E. 1978. Characteristics of Drosophila rhodopsin in wild-type and *norpA* vision transduction mutants. *J. Gen. Physiol.* 72:717–732.

34. Moore, W. J. 1962. Physical Chemistry. Prentice Hall, Englewood Cliffs, NJ.

35. Barlow, H. B. 1957. Purkinje shift and retinal noise. *Nature (Lond.).* 179:255–256.

36. Matthews, G. 1984. Dark noise in the outer segment membrane current of green rod photoreceptors from toad retina. *J. Physiol. (Lond.).* 349:607–618.