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PHYSIOLOGICAL CHARACTERISTICS OF SINGLE GREEN ROD PHOTORECEPTORS FROM TOAD RETINA

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SUMMARY

1. The outer segment membrane current of single isolated green and red rods from toad retina was recorded with a suction electrode, and characteristics of the response to light were examined.

2. The maximum response amplitude of green rods was smaller than that of red rods, but the density of dark current along the green rod outer segment was similar to previously reported values for red rods. Thus, the smaller maximum response is explained by the shorter outer segment of green rods (45 *vs.* 60 μm).

3. The intensity–response relation was fitted by a Michaelis equation with half-saturating photon density corresponding to about 55 isomerizations per flash.

4. The form of the green rod light response was similar to that of red rods: in both cases the kinetics were consistent with four first-order delay stages shaping the light response. The time-to-peak of the dim-flash response was usually about 1 sec for both green and red rods in the present experiments.

5. The spectral sensitivity curve of green rods was fitted by the nomogram for a vitamin A_1-based pigment with $\lambda_{max} = 433$ nm.

6. The relation between steady light intensity and flash sensitivity of green rods obeyed the Weber–Fechner relation, and the average background intensity necessary to reduce sensitivity to half of its dark level corresponded to about 4 isomerizations sec^{-1}. This is slightly lower than the value of about 8 isomerizations sec^{-1} reported for toad red rods by Baylor, Matthews & Yau (1980).

7. Green rods were similar to red rods in all respects except spectral sensitivity. Thus, no evidence was found to support the assertion that green rods are 'cone-like'.

INTRODUCTION

In amphibian retina, there are typically two types of rod photoreceptor, called 'red' and 'green' rods because of their characteristic colours when a flat mount of retina is viewed through a microscope in white light (Denton & Wyllie, 1955). There is some question whether the green rods might be more appropriately regarded as a type of cone photoreceptor, even though they are morphologically similar to rods. For example, behavioural evidence indicates that green rods are involved in hue discrimination in frogs (Muntz, 1962, 1963), and in e.r.g. experiments the photosensitivity of green rods has been reported to be lower than that of red rods (Frank,

1970; Witkovsky, Yang & Ripps, 1981). In addition, the visual pigment of green rods absorbs light maximally at a wave-length of 430–440 nm (Dartnall, 1967; Liebman & Entine, 1968; Harosi, 1975; Bowmaker & Loew, 1976), suggesting that green rods might be analogous to blue cones, which are absent in frog and toad retina. Further, the photoproducts are oriented axially after bleaching, as with cone pigments (Bowmaker, 1977). Although information about the electrophysiology of single green rods would be of value in determining their functional role, there is little such information. Most of what is known about green rods has come from studies of pigment extracts (Dartnall, 1967) or from microspectrophotometric studies of green rod outer segments (Liebman & Entine, 1968; Harosi, 1975; Witkovsky, Levine, Engbretson, Hassin & MacNichol, 1981). To my knowledge, the only published accounts of electrophysiological recordings from single green rods are two preliminary reports by Brown & Flaming (1977*a*, *b*) and a report of results from a single cell by Gold (1979). This is partly because the green rods make up about 10% of the total rod population and are thus encountered only rarely in physiological experiments employing intracellular recording.

In this paper, I report results of experiments on the physiological properties of single green rods from toad retina. Recordings of outer segment membrane current were made under visual control using the suction electrode technique recently developed by Baylor, Lamb & Yau (1979). Because red and green rods are morphologically distinct (Nilsson, 1964; Brown & Flaming, 1977*b*; Gold, 1981), it was possible to record preferentially from green rods, which were found to be similar to red rods in kinetics of the photocurrent, longitudinal density of dark current along the outer segment, half-saturating photon density at λ_{max}, and adaptation characteristics. The maximally absorbed wave-length was about 433 nm, in agreement with previously reported values from microspectrophotometry (Harosi, 1975).

METHODS

Experiments were performed on toads, *Bufo marinus*, obtained from commercial suppliers and kept in a large tank with free access to water and a dry platform warmed by a heat lamp. The light cycle was 12 hr on –12 hr off, and experiments were usually started at about the time lights would normally have been turned on. Animals were fed twice weekly with dog food and a multi-vitamin supplement. Before an experiment, an animal was dark-adapted overnight. Under dim red light, a toad was pithed and one eye was removed. The second eye was left in the carcass at room temperature for use 3–6 hr later. All subsequent manipulations were done using infra-red illumination and image converters. The retina was isolated and chopped into small pieces in oxygenated Ringer solution under a stereomicroscope equipped for infra-red viewing. Composition of Ringer solution was: NaCl, 111 mM; KCl, 2·5 mM; $CaCl_2$, 1·0 mM; $MgCl_2$, 1·6 mM; glucose, 10 mM; EDTA, 20 μm; HEPES, 3·0 mM; pH = 7·8. The pieces were transferred to a chamber constructed on a silanized glass slide, and the chamber was placed on the stage of an inverted compound microscope equipped with an infra-red-sensitive T.V. camera.

Isolated green and red rods were found lying among detached outer segments on the bottom of the chamber. Green rods could be readily distinguished from red rods by their shorter, thicker outer segments and thinner ellipsoid. Green rods usually broke off from the retina at the long, thin stalk connecting the myoid with the rest of the cell. Red rods broke off between the ellipsoid and the nucleus or between the nucleus and synaptic terminal. Recordings of outer segment membrane current were made as described by Baylor, Matthews & Yau (1980). Under visual control, the outer segment of an isolated cell was drawn into the tip of a glass micropipette connected to a current-to-voltage converter. In a few instances, recordings were made from the outer segment of

a cell attached to a small piece of retina. No difference was noted between responses of cells in pieces and isolated cells. To verify identification of a cell as a green or a red rod, sensitivity to 440 nm and 500 nm light was determined for each cell. Red rods, with λ_{max} near 500 nm (Harosi, 1975; Baylor *et al.* 1979), were more sensitive to 500 nm than 440 nm light, while green rods, with λ_{max} near 440 nm (Harosi, 1975; also see Fig. 6 of present paper), were more sensitive at 440 nm.

The amplified output of the current-to-voltage converter was recorded on a FM tape recorder (Precision Instruments PI-6200, band width 0–100 Hz, or Hewlett-Packard 3964 A, band width 0–312 Hz). Responses were usually replayed through an active low-pass filter (6-pole Butterworth; cut-off frequency 5–50 Hz), digitized at 256 points per response and 10–30 msec per point and analysed in a computer (Chrislin Industries CI–11/23–AC). Light stimuli were provided by a dual-beam optical stimulator similar to that described by Baylor & Hodgkin (1973). One beam gave 20-msec flashes whose wave-length and intensity were controlled by interference (Melles Griot, half-band width 10 nm) and neutral density filters. The second beam supplied infra-red illumination (Schott RG 850, $\lambda > 850$ nm) for viewing manipulations under the microscope. In experiments using flashes superimposed on background illumination, the second beam provided the background light ($\lambda = 420$ nm). All stimuli were unpolarized and applied diffusely (spot diameter = 600 μm). At the conclusion of each experiment, light intensity at each wave-length was measured by placing the sensing head of a calibrated photometer (United Detector Technology, Model 111 A) in the position normally occupied by the preparation.

RESULTS

Response amplitude as a function of intensity

The photocurrent of green rod outer segments was similar to that of red rods (Baylor *et al.* 1979). Fig. 1 *A* shows superimposed responses of a green rod to 440 nm flashes of increasing intensity; for comparison, responses of a red rod to 500 nm flashes are shown in Fig. 1 *B*. The relation between normalized peak response and flash photon density is plotted in Fig. 1 *C* for the green rod and in Fig. 1 *D* for the red rod. In both cases the continuous line through the data points is drawn from the Michaelis equation,

$$r/r_{max} = i_f/(i_f + i_h), \quad (1)$$

where r is peak response amplitude, r_{max} is maximum response amplitude, i_f is flash photon density, and i_h is half-saturating intensity. Values of i_h were 1·4 440 nm photons μm^{-2} for the green rod and 1·4 500 nm photons μm^{-2} for the red rod of Fig. 1. Results of experiments similar to those shown in Fig. 1 are summarized in Table 1. The half-saturating intensity, estimated by fitting eqn. (1) to the data, averaged $2{\cdot}38 \pm 1{\cdot}3$ 440 nm photons μm^{-2} for green rods (mean $\pm$ S.D. $N = 13$) and $1{\cdot}29 \pm 0{\cdot}38$ 500 nm photons μm^{-2} for red rods (mean $\pm$ S.D. $N = 5$). Using light plane-polarized perpendicular to the long axis of the outer segment, Baylor *et al.* (1979) reported i_h of 1·46 500 nm photons μm^{-2} for toad red rods. Assuming a dichroic ratio of 4·06 (Harosi, 1975), this corresponds to 2·34 photons μm^{-2} for unpolarized light, similar to the value obtained for green rods in the present experiments.

If the Michaelis relation provides a satisfactory description of the response–intensity relation, it follows from eqn. (1) that i_h can be estimated from the relation $i_h = r_{max}/S_f$, where r_{max} is the saturated response amplitude and S_f is the flash sensitivity for dim flashes, defined as peak response amplitude divided by flash photon density. Values of i_h estimated in this way are also presented in Table 1. The average was $2{\cdot}62 \pm 1{\cdot}58$ 440 nm photons μm^{-2} for twenty green rods and $1{\cdot}38 \pm 0{\cdot}44$ 500 nm photons μm^{-2} for eight red rods, similar to the estimates obtained from complete flash families.

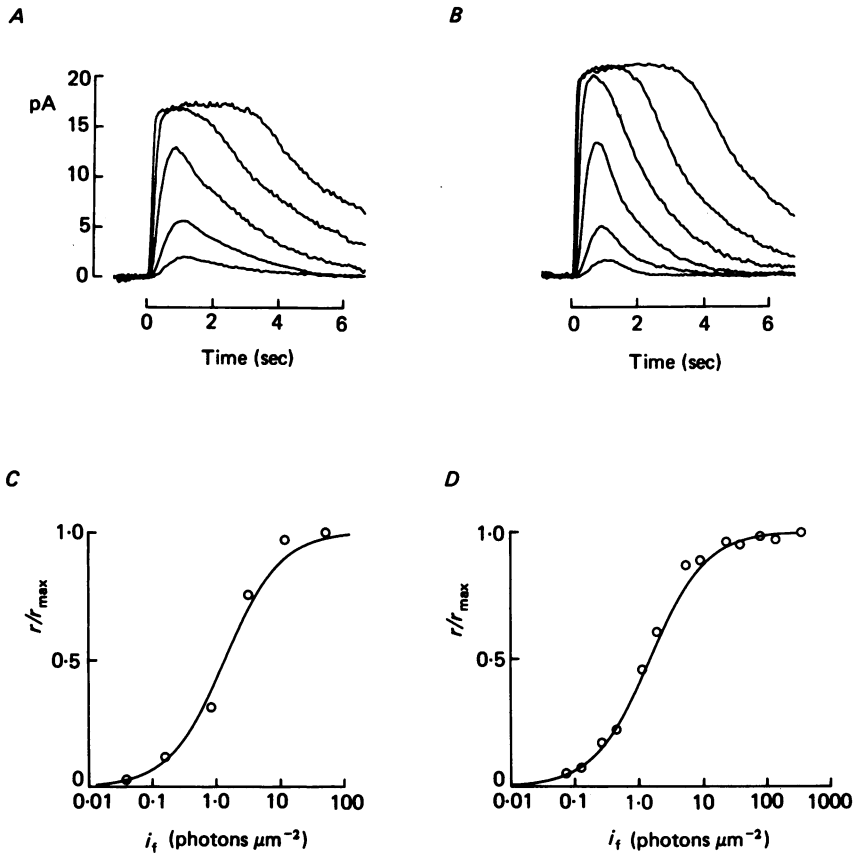

Fig. 1. Responses of a green and red rod to flashes of increasing intensity. *A*, superimposed responses of a green rod. Responses are change in membrane current from its dark level, with outward current plotted upward. Flash intensities in 440 nm photons μm^{-2}, with number of responses averaged in parentheses, were: 0·157 (15), 0·818 (27), 3·53 (3), 12·5 (2), 51·2 (1). Flashes (20 msec) were delivered at time 0. Low-pass filtered at 10 Hz. Temperature 22·3 °C. *B*, superimposed responses of red rod. Flash intensities in 500 nm photons μm^{-2}, with number of responses averaged in parentheses, were: 0·125 (14), 0·443 (10), 1·88 (6), 9·00 (3), 38·1 (3), 135 (3). Flash (20 msec) delivered at time 0. Low-pass filtered at 10 Hz. Temperature 21·6 °C, semi-logarithmic plot of peak amplitude of responses of cell in *A* against flash intensity. The continuous line is drawn according to the Michaelis relation (eqn. (1)), with $i_h = 1{\cdot}4$ 440 nm photons μm^{-2}. *D*, semi-logarithmic plot of peak amplitude of responses of cell in *B* against flash intensity. For clarity, some intensities plotted in *D* were not shown in *B*. The continuous line is eqn. (1) with $i_h = 1{\cdot}4$ 500 nm photons μm^{-2}.

Response–intensity relations for nine green rods are summarized in Fig. 2. Data were normalized by expressing flash intensity relative to the value of i_h for each cell. Equation (1) provided a reasonable fit to the data, although the observed relation usually rose slightly more rapidly than predicted from the Michaelis equation. This has been noted before for red rods (Baylor *et al.* 1979; Lamb, McNaughton & Yau, 1981), and an explanation in terms of the spatial spread of excitation along the outer segment has been proposed (Lamb *et al.* 1981).

TABLE 1. Summary of half-saturating intensity for red and green rods

	Cell	i_h (photons μm^{-2})	$r_{max} S_f$ (photons μm^{-2})
Green rods	1	2·19	2·74
	2	4·60	—
	3	5·00	7·71
	4	1·63	—
	5	—	1·67
	6	—	2·29
	7	2·05	2·10
	8	1·00	1·13
	9	3·15	3·45
	10	2·20	2·41
	11	2·05	2·73
	12	1·72	2·42
	13	0·66	0·80
	14	—	0·78
	15	—	0·95
	16	1·40	1·32
	17	—	3·33
	18	—	4·20
	19	3·25	2·38
	20	—	4·19
	21	—	2·76
	22	—	3·12
Red rods	1	—	1·69
	2	0·61	0·88
	3	—	0·58
	4	1·53	1·93
	5	1·30	1·58
	6	1·60	1·47
	7	1·40	1·40
	8	—	1·51

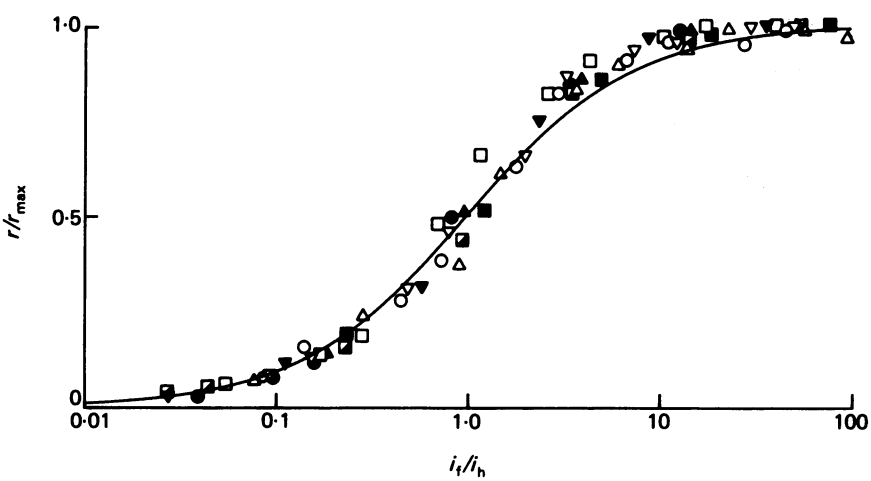

Fig. 2. Compiled results of flash families like those of Fig. 1*C* for nine green rods. Each symbol shows the results from a different cell. Flash intensity was normalized with respect to the value of i_h obtained by fitting eqn. (1) to data for each cell. Continuous line is drawn according to the Michaelis relation (eqn. (1)).

Density of dark current along outer segment

The maximum amplitude of the photocurrent of green rods was consistently less than that of red rods (cf. Fig. 1 *A* and *B*). The largest saturating response observed was 17·2 pA for green rods, and most were between 10 and 12 pA. Isolated red rods, however, frequently had saturating responses greater than 20 pA. This differences in r_{max} would be expected if the longitudinal density of dark current along the outer segment were the same for green and red rods. Because the green rod outer segment is shorter (45 *vs.* 60 μm), a smaller r_{max} for green rods would result. To test this possibility, current density was measured along the outer segment of green rods.

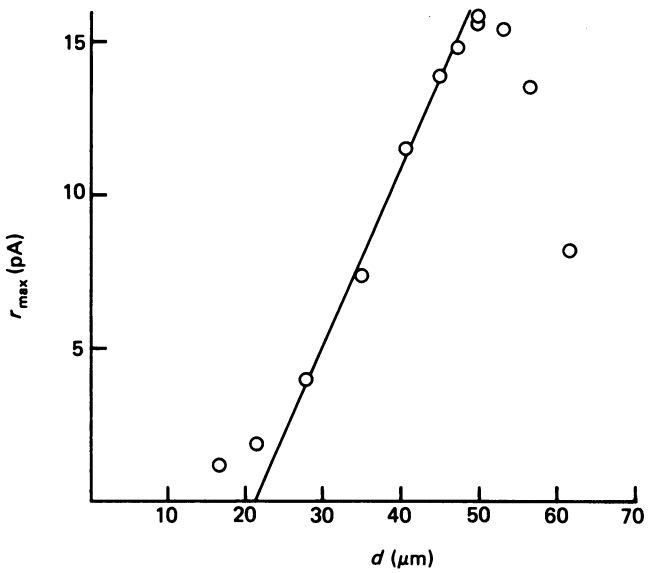

Fig. 3. Relation between maximum response amplitude and length of outer segment within suction electrode. Isolated green rod. Length within electrode (d) was measured from the outside edge of the tip of the suction electrode. The total length of the outer segment was 46·6 μm. A straight line with slope 0·575 pA μm^{-1} was drawn through the region over which maximum response rose linearly with recorded length.

Varying lengths of the outer segment were drawn into the recording electrode, and the maximum light response was measured at each position. Because r_{max} is equal and opposite to the dark current (Baylor *et al.* 1979), this procedure gives the magnitude of the dark current as a function of recorded length of outer segment, and the slope of this relation at a point gives the dark current density at that point. Results of one such experiment are shown in Fig. 3. As the outer segment was drawn into the electrode, the dark current at first increased slowly as the outer segment filled the constriction and the seal resistance increased. When about 20 μm of outer segment had been drawn into the electrode, the constriction was fully occupied and the seal resistance had reached its maximum value; after that point, the dark current rose approximately linearly with recorded length, with a slope of 0·575 pA μm^{-1} for the cell of Fig. 3. Eventually, the inner segment began to enter the electrode, and the recorded dark current declined as the return path for the dark current fell within

the electrode. In eight such experiments, the density of current along the outer segment averaged $0{\cdot}54 \pm 0{\cdot}07$ pA μm^{-1} (mean $\pm$ S.D.). This is similar to the value of $0{\cdot}54 \pm 0{\cdot}09$ pA μm^{-1} reported for toad red rods by Baylor *et al.* 1979), suggesting that the smaller response maxima observed for green rods were due to their shorter outer segments.

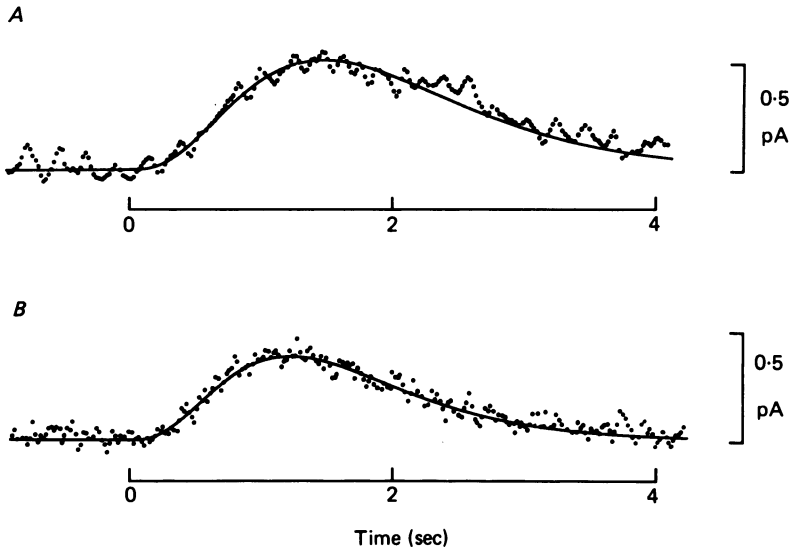

Fig. 4. Kinetics of dim-flash responses of green and red rods. *A*, average response of a green rod to forty 20 msec flashes of intensity 0·0384 440 nm photons μm^{-2}, delivered at time 0. Low-pass filtered at 10 Hz. The continuous line through the data points is eqn. (2) (the Poisson relation) with $n = 4$ stages and $\alpha = 2{\cdot}06$ sec^{-1}. Temperature 21·2 °C. *B*, average response of red rod to forty 20 msec flashes of intensity 0·0586 500 nm photons μm^{-2}. Low-pass filtered at 20 Hz. Continuous line is eqn. (2) with $n = 4$ stages and $\alpha = 2{\cdot}48$ sec^{-1}. Temperature 23·0 °C.

Response kinetics

The kinetics of the light response of toad red rods are consistent with a series of four first-order delays shaping the change in membrane current (Baylor *et al.* 1979; Baylor *et al.* 1980). Because these delays may represent stages in the transduction process in the outer segment, it is important to determine the generality of this scheme in different photoreceptors. For that reason, the kinetics of the photocurrent of green rods were examined in two ways: (1) by fitting the Poisson and Independence expressions of Baylor *et al.* (1979) to the average dim-flash response and (2) by determining the limiting slope on double logarithmic co-ordinates of the initial rise of responses to flashes of various intensities. An example of the averaged dim-flash response of an isolated green rod is shown in Fig. 4*A*. For comparison, the response of a red rod is shown in Fig. 4*B*. In each case, the smooth curve through the response is the Poisson expression of Baylor *et al.* (1979):

$$r(t) = ik\,(\alpha t)^{n-1}\,e^{-\alpha t}, \tag{2}$$

where $r(t)$ is the photocurrent following the flash, i is flash intensity and k is a sensitivity constant. This expression gives the impulse response of a series of n

buffered low-pass filters with equal rate constant, α. For both the red and green rod of Fig. 4, four stages of delay provided the best fit to the dim-flash response. The independence relation of Baylor *et al.* (1979) sometimes provided a better fit than eqn. (2). This expression is given by

$$r(t) = ike^{-\alpha t}(1-e^{-\alpha t})^{n-1} \quad (3)$$

and gives the impulse response of a series of n buffered low-pass filters with rate constants α, 2α, 3α, ..., $n\alpha$. In fifteen cells whose dim-flash responses were fitted by eqn. (2) or eqn. (3), the number of stages, n, was consistently four. In a few

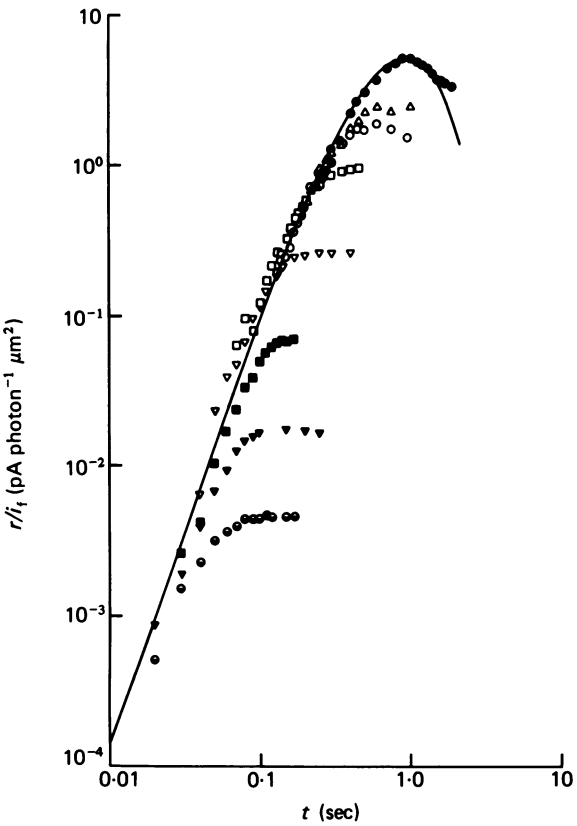

Fig. 5. Double-logarithmic plot of response amplitude divided by light intensity against time after a 20 msec flash for a single isolated green rod. Each symbol shows the results for a different flash intensity. Intensity was increased in steps of approximately 0·6 log unit from 0·148 to 2800 440 nm photons μm^{-2}. The continuous line is eqn. (2) with $n = 4$ and $\alpha = 3{\cdot}12$ sec^{-1}. Responses are averages of from 2 to 50 sweeps. Responses to dimmest flash were low-pass filtered at 5 Hz; responses to five brightest flash intensities were low-pass filtered at 50 Hz. Other responses were low-pass filtered at 20 or 30 Hz.

instances, the rising phase of the dim-flash response could be fitted adequately by eqn. (2) or eqn. (3) with $n = 4$, but the falling phase was more prolonged than would be expected from either the Poisson or Independence expression.

The number of delay stages shaping the light response can also be estimated from double-logarithmic plots of response size divided by flash intensity against time after

the flash, for flashes of different intensities. In this way the response can be analysed at early times after the flash by making use of the small linear portion at the beginning of bright-flash responses. If four delay stages shape the light response, it would be expected that the rise of the response at early times should have a limiting slope of three on double-logarithmic co-ordinates. An example of such a plot for eight different flash intensities is shown in Fig. 5. The continuous line is eqn. (2) with $\alpha = 3{\cdot}12$ sec^{-1} and $n = 4$. The rising phase of the response had a slope of three, as expected for four stages of delay shaping the light response. Similar results were seen in ten other green rods; in all cases the initial rise of the light response indicated four delay stages.

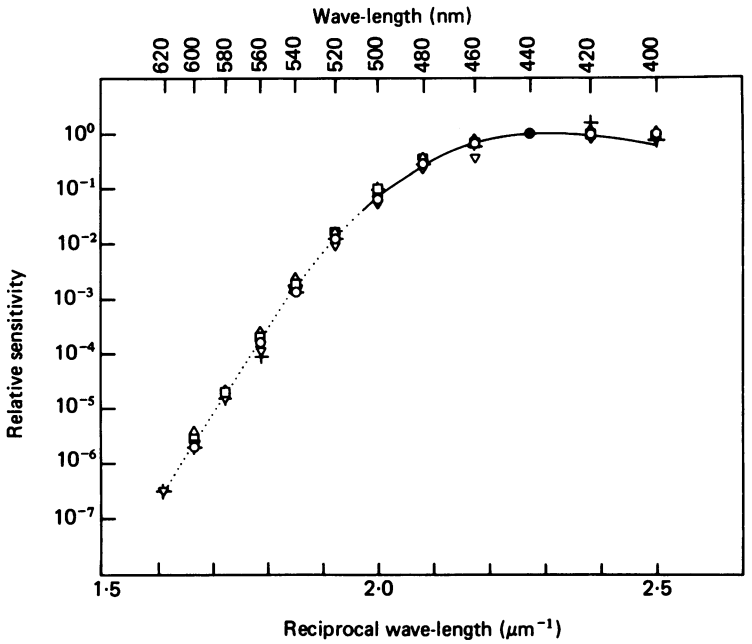

Fig. 6. Spectral sensitivity of green rods. Compiled results from five cells. Results were combined by normalizing with respect to the sensitivity of each cell at 440 nm. The continuous line is the nomogram for a vitamin A_1-based pigment with $\lambda_{max} = 433$ nm (from Fig. 17 of Knowles & Dartnall, 1977).

Spectral sensitivity

Green rods absorb light maximally at wave-lengths between 430 and 440 nm (Dartnall, 1967; Liebman & Entine, 1968; Harosi, 1975; Bowmaker & Loew, 1976). In order to determine the spectral sensitivity over a wider sensitivity range than can be obtained with microspectrophotometry of isolated outer segments or with spectrophotometry of pigment extracts, spectral sensitivity was studied electrophysiologically. In these experiments, sensitivity was determined in one of two ways. In the first, the sensitivity at a particular wave-length was defined as the reciprocal of the half-saturating light intensity obtained by fitting a Michaelis relation to a partial response–intensity relation. In the second method, sensitivity at each wave-length was estimated from average response amplitude divided by flash photon density for flashes producing small, linear responses. Results from the two methods were indistinguishable and are summarized for five green rods in Fig. 6. To allow

combination of the results from different cells, sensitivity is expressed relative to its value at 440 nm for each cell. The continuous line in the Figure is the nomogram (Knowles & Dartnall, 1977) for a vitamin A_1-based pigment with λ_{max} of 433 nm. The green rod nomogram of Ebrey & Honig (1977) was also fitted to the data (not shown in Fig. 6) and gave a similar value for λ_{max}. The data of Fig. 6 agree well with those of Harosi (1975), whose microspectrophotometric experiments yielded a λ_{max} of 433 nm for toad green rod outer segments. The dotted line in Fig. 6 was drawn by eye and may represent the extension of the nomogram to wave-lengths longer than

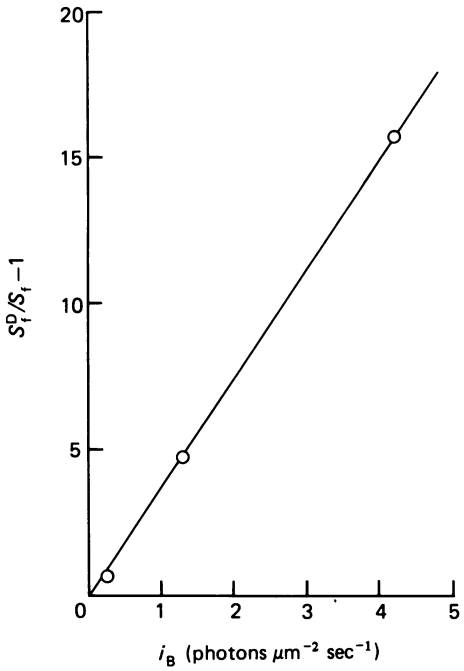

Fig. 7. Sample results showing effect of background illumination on flash sensitivity of a green rod. Wave-lengths of background and test flashes were 420 and 440 nm, respectively. If the Weber–Fechner relation (eqn. (4)) is obeyed, the results should fall on a straight line passing through the origin when the parameter $S_f^D/S_f - 1$ is plotted against background light intensity, i_B. For this cell, the slope of the straight line through the data points corresponds to a half-desensitizing background intensity, i_0, of 0·27 420 nm photons μm^{-2} sec^{-1}.

500 nm. The terminal slope of the dotted line was $1{\cdot}21 \times 10^{-13}$ sec, or 0·74 (h/kT), where h is Planck's constant, k is Boltzmann's constant and T is the absolute temperature. This is similar to the asymptotic slope of 0·78 (h/kT) for the fall-off of human rod sensitivity at long wave-lengths (Rodieck, 1973).

Effect of background light on flash sensitivity

It has been suggested that green rods are similar to cones in some respects. If this is the case, it is possible that green rods may differ from red rods in adaptation to steady illumination. To examine this, flash sensitivities of green rods were tested in darkness and in the presence of steady illumination of varying intensity. In these

experiments, 440 nm flashes were superimposed on steady 420 nm background lights. It has been shown previously that in such an experiment the relation between background light intensity and flash sensitivity of toad red rods obeys the Weber–Fechner relation (Bastian & Fain, 1979; Baylor *et al.* 1980):

$$S_f/S_f^D = 1/(1+i_B/i_0) \quad (4)$$

The background light intensity, i_0, that reduced flash sensitivity (S_f) to half of its value in darkness (S_f^D), was about 0·18 500 nm photons μm^{-2} sec^{-1} for red rods. Sample results from an adaptation experiment on a green rod are shown in Fig. 7, which shows $S_f^D/S_f - 1$ plotted against background light intensity, i_B. Plotted in this manner, eqn. (4) appears as a straight line of slope $1/i_0$ passing through the origin (Lamb *et al.* 1981). For the cell of Fig. 7, eqn. (4) provided a good fit to the data with i_0 of 0·27 420 nm photons μm^{-2} sec^{-1}. In eleven experiments, i_0 averaged 0·176 ± 0·093 420 nm photons μm^{-2} sec^{-1}. (mean ± S.D.), similar to the value previously reported for red rods.

Green rods might also differ from red rods in recovery of sensitivity following light-induced desensitization. In experiments to examine this, recovery of sensitivity following a steady, saturating light was studied in red and green rods. The intensity of the steady light was adjusted for each cell to just maintain saturation for the duration of illumination. The duration of exposure ranged from 30 to 70 sec, following which periodic dim flashes were given to assess the return of flash sensitivity to its original level. No difference was noted in the rate of recovery of red and green rods under these conditions. In four experiments on green rods, sensitivity recovered along an approximately exponential time course with time constant ranging from 17·6 to 25·2 sec. In two experiments on red rods, the time constants were 18·7 and 23·7 sec.

DISCUSSION

No support was found for the notion that green rods are functionally more like cones than rods. In particular, the photon sensitivity at the maximally absorbed wave-length was similar for green rods and red rods. The half-saturating flash intensity in the present experiments averaged about 2·4 440 nm photons μm^{-2} for green rods. In order to translate this flash photon density into photo-isomerizations, the effective collecting area, A, can be estimated from the relation

$$A = 2{\cdot}303\pi\, r^2 lqfd \quad (5)$$

where r and l are outer segment radius and length, q is the quantum efficiency of isomerization (0·67 for rhodopsin; Dartnall, 1972), f is a factor that depends on the polarization of the light and D is the specific axial pigment density (0·014 μm^{-1} for toad green rods; Harosi, 1975). For unpolarized light $f = 0{\cdot}62$, assuming a dichroic ratio of 4·06 (Harosi, 1975). Green rod outer segments are about 7 μm in diameter and about 45 μm long, giving an effective collecting area of about 23 μm^2 in the present experiments. Thus, the average half-saturating intensity for green rods corresponds to approximately 55 photo-isomerizations per flash. Baylor *et al.* (1979) reported an average half-saturating intensity of 1·46 photons μm^{-2} for red rods, corresponding to about 60 photo-isomerizations per flash (assuming an effective

collecting area of about 42 μm^{-2} for light plane-polarized normal to the long axis of the outer segment). In the present experiments, the average half-saturating intensity of 1·29 photons μm^{-2} for red rods was somewhat less than that reported by Baylor *et al.* (1979), and gave an estimate of 33 photo-isomerizations per flash at half-saturation. Nevertheless, it is clear that green and red rods are comparably sensitive to light at their respective maximally absorbed wave-length.

To compare the effect of background illumination on the flash sensitivity of red and green rods the steady light, i_0, necessary to reduce flash sensitivity to half its dark value can also be expressed in photo-isomerizations sec^{-1}. For green rods, the average value of i_0 was about 0·18 photons μm^{-2} sec^{-1}, corresponding to about 4 photo-isomerizations sec^{-1}. Under similar experimental conditions, Baylor *et al.* (1980) reported that i_0 produced about 8 photo-isomerizations sec^{-1} in toad red rods. Thus, green rods are desensitized by steady illumination in a manner similar to red rods.

The green rod light response could be described by the same expressions that fit the red rod light response (Baylor *et al.* 1979, 1980). In both cases, the form of the light response was consistent with a series of four first-order delays shaping the change in membrane current. This quantitative similarity suggests that the scheme for phototransduction suggested for red rods by Baylor *et al.* (1980) and Matthews & Baylor (1981) might be generally applicable. The observation that the light response of rat rods is also fitted by four stages of delay (Penn & Hagins, 1972) adds further weight to this possibility.

The similarity between red and green rods raises again the question of the role of the green rods in amphibian vision. The present experiments have established the physiological characteristics of green rod photoreceptors but provide no information about their adaptive value for the animal. Speculations about that role may be divided into two broad classes: (1) that they act in conjunction with the red rods to give limited hue discrimination at dim light levels; and (2) that they play the role of blue cones in trichromatic colour vision in an environment in which light in the blue end of the visual spectrum is attenuated. The latter environment might arise, for example, in turbid water, in which short wave-length light would scatter more than longer wave-lengths and would thus not penetrate as deeply.

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