Journal of Physiology (1993), **471**, *pp.* 817–829
With 5 figures
Printed in Great Britain

PHOTOCURRENTS IN RETINAL RODS OF PIGEONS (*COLUMBA LIVIA*): KINETICS AND SPECTRAL SENSITIVITY

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(*Received 24 November 1992*)

SUMMARY

1. Membrane photocurrents were recorded from outer segments of isolated retinal rods of pigeons (*Columba livia*), the first such measurements on the photoreceptors of a bird. The amplitude of the response to 20 ms flashes of narrow wavelength bands of light increases linearly with intensity at low photon fluxes and saturates at higher intensities. The maximum (saturating) photocurrent observed in forty-nine rod cells was 50 pA. Larger responses with less variability in the intensity for half-maximal responses were observed when the physiological saline contained 20 mM bicarbonate (in addition to Hepes buffer).

2. The dependence of peak amplitude on intensity is well fitted by an exponential function; it is usually less well fitted by the Michaelis–Menten (Naka–Rushton) equation.

3. In the presence of bicarbonate, the average sensitivity of pigeon rods to dim flashes was 0·56 pA photon^{-1} μm^{-2}. The effective collecting area per photon was 1·8 μm^2. About 83 $\pm$ 26 (mean $\pm$ S.D.) photoisomerizations were required for a half-saturating response.

4. The response kinetics of rods to dim flashes can be reasonably well described by a series of four to five either Poisson or independent filters. The time to peak, measured from the mid-point of a 20 ms flash, was 319 $\pm$ 83 ms (mean $\pm$ S.D.). The integration time of the response was 851 $\pm$ 86 ms (mean $\pm$ S.D.) with bicarbonate present and 572 $\pm$ 126 ms in the absence of bicarbonate. The responses of pigeon rods appear to be slower than those of mammals at the same temperature. The fraction of current suppressed by a single photoisomerization is smaller in pigeon than in mammalian rods by a factor of at least two.

5. The spectral sensitivity function was measured between 680 and 330 nm. The maximum at about 505 nm (range 497–508 nm) corresponds to the α-band of a vertebrate rhodopsin and agrees with previous behavioural measurements of scotopic sensitivity of pigeons as well as the absorption spectrum of extracts of pigeon rhodopsin. There was no pronounced β-band in the near-ultraviolet wavelengths.

INTRODUCTION

Recent studies of retinal phototransduction pursued in vertebrate photoreceptors at both physiological and molecular levels have provided much information on spectral sensitivity, kinetics of response, the cGMP cascade, the

nature of receptor noise, and the processes of adaptation (for reviews see Stryer, 1986; Yau & Baylor, 1989; McNaughton, 1990; Nakatani, Tamura & Yau, 1991; Lagnado & Baylor, 1992). In birds, however, where diverse lines of evidence suggest the presence of more visual pigments than are present in primates (Goldsmith, 1990; Bowmaker, 1991), with a correspondingly complex visual system (Varela, Palacios & Goldsmith, 1993), there is little information available on the responses of photoreceptors. This paper provides a start by describing the photocurrent responses of pigeon rods. A preliminary presentation of some of these data has appeared elsewhere (Palacios & Goldsmith, 1992).

METHODS

The basic experimental method is a modification of the technique of Baylor, Lamb & Yau (1979*a*) and Baylor, Nunn & Schnapf (1984) for recording membrane photocurrents from isolated retinal photoreceptors of vertebrates.

Animals

Pigeons, *Columba livia*, were obtained locally and housed with free access to food and water for up to 3–4 weeks before being used for an experiment. Animals were dark adapted for 2–3 h, sometimes overnight, and anaesthetized with an intramuscular injection of Ketalar (Parke Davis, USA; 40 mg kg^{-1} body weight) before decapitation. The eyes were removed under deep red light, and all subsequent steps performed under infrared light, observing with an infrared-sensitive TV camera. The cornea was removed and the eye incubated for 1–2 h in tissue culture medium (199, or occasionally L-15; Gibco, Gaitherburg, MD, USA) at 1–3 °C before attempting to remove the retina. The pre-incubation proved necessary in order to separate the retina from the pigment epithelium and vitreous humour, which in pigeons is very viscous and adheres to the retina (Rochon-Duvigneaud, 1943; Cohen, 1963). The retina was transferred to plastic culture dishes with 4 ml of medium 199 supplemented with one drop of a mixture of penicillin and streptomycin (5000 units ml^{-1} and 5000 μg ml^{-1}, Gibco). The retina was then maintained at 2–3 °C in a light-tight box. The photoreceptors remained healthy for more than 36 h under these conditions, and better isolation of receptors was achieved after the first 12 h. A similar observation was made in the retina of *Bufo* (Leibovic, 1986).

Pieces of retina were diced into small fragments on a substrate of Sylgard (Dow Corning, Medfield, MA, USA) or mechanically dissociated by trituration with a fire-polished Pasteur pipette and transferred to the recording chamber on the stage of the microscope. Photocurrents were usually recorded from photoreceptors attached to a small piece of retina. A few records were obtained from isolated rods found in the bottom of the chamber ($n = 5$) or from rods obtained by 30 min enzymatic dissociation with papain (Sigma, St Louis, MO, USA; P-4762, 12 units ml^{-1}, pre-activated with crystals of L-cysteine, Sigma, C-7880) at 2 °C ($n = 4$). No differences were found with any of these methods, all of the data have been used for analysis, but only four of these nine cells appear in Table 1. Although pigeon rods are similar in size to cones, they were readily identified for recording by the absence of an oil droplet. Their identification was subsequently confirmed by their kinetics of response and their spectral sensitivity (Results).

Solutions

The original physiological saline solution in which the retina was placed for recording consisted of (mM): NaCl, 120; $MgCl_2$, 1·2; KCl, 3·6; $CaCl_2$, 2·4; glucose, 10; EDTA, 0·02; and Hepes, 3, buffered to pH 7·4 with NaOH. In later experiments the solution also contained 20 mM bicarbonate, resulting in somewhat larger saturating responses and considerably less variability in the intensity of light required for half-saturating responses (i_0, Table 1). The solution was aerated with 95 % O_2– 5 % CO_2; in the absence of added bicarbonate, final pH was 7·2.

Recording chamber

The recording chamber consisted of two microscope coverglasses (the upper of quartz) separated by a plastic frame, closed on three sides, and mounted on the stage of an inverted

microscope (Leitz Diavert, Wetzlar, Germany). The temperature of oxygenated Ringer solution was adjusted in the chamber to 39 °C, the approximate temperature of the pigeon's brain (Arad, 1989), using a small heating coil and monitoring with a calibrated thermistor (Victory Engineering, Springfield, NJ, USA). The superfusate was drawn from the open front of the chamber with a small tube to which negative pressure was applied.

For recording, outer segments were drawn into the fire-polished tip of a silanized (Sigmacote, Sigma, USA) pipette with gentle suction until there was a 4- to 6-fold increase in electrical resistance. The electrodes were calomel half-cells. After current-to-voltage conversion the membrane response was filtered (active, 20 Hz low-pass, four-pole Bessel filter), and monitored on an oscilloscope. At this frequency setting, the filter introduced a 20 ms delay (empirically determined), and the reported temporal parameters in Table 1 have been correspondingly corrected. The timing, wavelength, and intensity of the stimulating light as well as acquisition of data were managed by a computer (Intel 80386 processor, Northgate, Plymouth, MN, USA) fitted with a Dash-8 (Metrabyte, Keithley, Taunton, MA, USA) interface card containing a 12 bit A–D converter capable of sampling at 4 kHz. Data were sampled continuously and averaged into 10 ms bins, yielding 100 data points per second.

Optical system

The unpolarized stimulus beam was provided by a 150 W xenon arc lamp operated from a stabilized power supply (Ealing, Holliston, MA, USA). A grating monochromator (Bausch & Lomb, Rochester, NY, USA; 600 lines mm^{-1} grating) was used to obtain narrow (10 nm half-bandwidth) stimuli between 330 and 680 nm. A long-pass gelatin filter (no.16, Kodak Wratten, Rochester, NY, USA; transmission < 1 % at wavelengths < 510 nm) was used to remove stray light and the second-order spectrum when the monochromator was set to wavelengths longer than 600 nm.

A 500 μm diameter spot was focused on the bottom of the recording chamber. The intensity was controlled with a pair of counter-rotating optical wedges, each consisting of a graded film of inconel deposited in an annulus on a disc of quartz. The duration of stimulating flashes was controlled with an electronic shutter (Uniblitz, Vincent Associates, Rochester, NY, USA). A second light (100 W tungsten lamp) fitted with infrared (IR) filters (optical density > 4 at wavelengths shorter than 750 nm) was combined with the stimulating beam so that the preparation could be observed with an IR-sensitive video camera and television monitor.

The xenon lamp and optical wedges were calibrated with a photodiode of known sensitivity (United Detector Technology, Hawthorne, CA, USA) connected to a digital electrometer (Keithley 616, Taunton, MA, USA). A holmium oxide filter was used to calibrate the wavelength of the monochromator.

Collecting area

The effective collecting area of a rod (A_c) was estimated to be $1{\cdot}8 \pm 0{\cdot}45$ μm^2 (mean ± S.D.), from

$$A_c = 2{\cdot}303\, D\pi\rho^2\zeta\gamma f, \tag{1}$$

where D is the absorbance per micrometre at the λ_{max}, ρ and ζ are the radius and length of the outer segment in the recording pipette, γ is the quantum efficiency of isomerization, and f is an orientation factor which depends on the polarization of the measuring beam, its relation to the disc membranes of the outer segment, and the dichroic ratio of the pigment (Baylor, Lamb & Yau, 1979*b*). D was taken to be $0{\cdot}018$ μm^{-1} (Bowmaker, 1977); ρ and ζ, 1 and 31 ± 8 μm (mean ± S.D., seventeen cells measured on the monitor during experiments); and γ, 0·67 (Dartnall, 1972). The polarization of the stimulus beam was measured as a function of wavelength, and assuming a dichroic ratio of about 4, similar to that in other vertebrate rods (Harosi, 1975; Baylor *et al.* 1984), f at 500 nm was about 0·66.

Integration time

The integration time was determined from

$$t_i = \int r(t)\mathrm{d}t / r_{peak}, \tag{2}$$

where $r(t)$ is the response to a dim flash and r_{peak} the peak amplitude (Baylor & Hodgkin, 1973).

Spectral sensitivity

The reciprocal of the quantal flux required to produce a photocurrent of constant amplitude as a function of wavelength was measured by recording responses of near-criterion amplitude, fitting the data to a segment of a response–energy function, and determining the criterion response, and thus the required energy, by interpolation. The response sensitivity to a 500 nm light was used as reference value and measured several times during an experiment in order to correct for any drift in sensitivity. The sensitivities at other wavelengths were plotted relative to the reference value.

RESULTS

Shape of response to brief flashes

Figure 1 shows a family of rod responses to 20 ms flashes of light of increasing intensity. The traces, plotted relative to a baseline dark level, show progressive suppression of the dark current. The saturating response (r_{max}), which represents complete suppression of the circulating dark current, was about 20 pA in this cell.

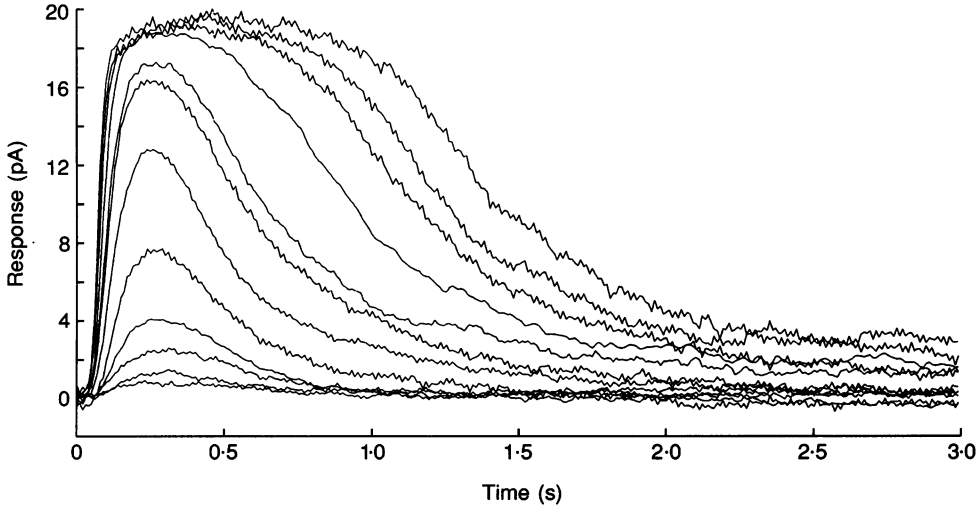

Fig. 1. Family of responses of an isolated pigeon rod to 20 ms flashes of 500 nm light of increasing intensity. The dimmest flash was 8·5 photons μm^{-2}, and successive flashes were 17, 30, 60, 120, 240, 380, 600, 960, 1520, 1900 and 2400 photons μm^{-2}. Individual curves are an average of two to six responses. Temperature was 39 °C; bandpass was 0–20 Hz. Cell 1 in Table 1.

The mean r_{max} for forty-two rods in the absence of bicarbonate was 15 pA (range, 6–35 pA). In the presence of bicarbonate, mean r_{max} for seven rods was 33 pA (range, 20–50 pA). The time to peak varied with the flash intensity, being 25 % shorter with just-saturating intensities than with dim flashes near threshold. Saturating responses were followed by a long tail of photocurrent of 2–5 pA in amplitude. Details for seventeen cells with complete data and $r_{max} > 10$ pA are given in Table 1.

TABLE 1. Summary of responses of seventeen pigeon rods

Cell	r_{max}	t_{peak}	t_1	τ	F	i_0	S_ϕ/r_{max} (× 100)
1	20	286	548	72	I (5)	165	0·30
2	18	286	582	72	I (5)	13	2·67
3	28	379	498	126	P (4)	54	0·68
4	12	326	629	81	I (5)	147	0·42
5	10	148	275	37	I (5)	56	0·80
6	15	362	627	121	P (4)	105	0·20
7	16	416	735	139	P (4)	37	1·38
8	11	342	742	86	I (5)	30	0·64
9	10	276	563	69	I (5)	45	0·70
10	21	368	523	92	P (5)	105	0·81
11	11	273	574	68	I (5)	34	0·64
Mean	16	315	572			72	0·84
S.D.	5·8	73	126			51	0·68
12	30	280	856	93	I (4)	55	1·17
13	23	531	881	177	I (4)	36	1·04
14	42	280	817	70	I (5)	57	0·86
15	30	350	725	117	I (4)	51	0·80
16	36	290	988	97	I (4)	50	2·08
17	20	230	836	77	I (4)	57	1·30
Mean	30	327	851	105		51	1·21
S.D.	8·1	107	86	39		8	0·47

Upper block, Ringer solution without bicarbonate; lower block, 20 mM bicarbonate present.

Symbols: r_{max}, peak amplitude of maximum (saturating) photocurrent (pA); t_{peak}, time to peak (ms); t_1, integration time (ms; eqn (2)); F, filter model, Poisson (P) or independent (I) (number of stages, n, for best fit; eqns (6) and(7)); τ, $t_{peak}/(n-1)$, time constant in kinetic models; i_0, intensity for half-maximal response (photons μm^{-2}); $S_\phi/r_{max} \times 100$, percentage of the current suppressed by single photoisomerizations (linear range).

Cells 1–11 are a subset (maximal response > 10 pA) of rods recorded in physiological saline buffered with Hepes; cells 12–17 were recorded in the same saline but with the addition of 20 mM bicarbonate (details in Methods).

Response–intensity function

Figure 2 shows the peak amplitude of rod responses to flashes of increasing intensity at 500 nm for the cell in Fig. 1 (■) and four others. Similar results were obtained with other wavelengths (data not shown). The continuous curve is the best fit of the following exponential function (Lamb, McNaughton & Yau, 1981):

$$r = r_{max}[1 - \exp(-k_f i)], \tag{3a}$$

where r is the peak rod response, r_{max} is the amplitude of the saturating response at the peak, k_f is a coefficient characteristic of the cell, and i is the number of quanta per square micrometre. For most cells, this function provided a better fit to the data than the Michaelis–Menten formulation (Fig. 2, dashed curve), where

$$r = r_{max}\,[i/(i + i_0)]. \tag{3b}$$

The number of photons required for a half-saturating response (i_0) was determined for each cell by fitting the data to either eqns (3a) or (3b), whichever gave the smaller sum of squared differences between data and function.

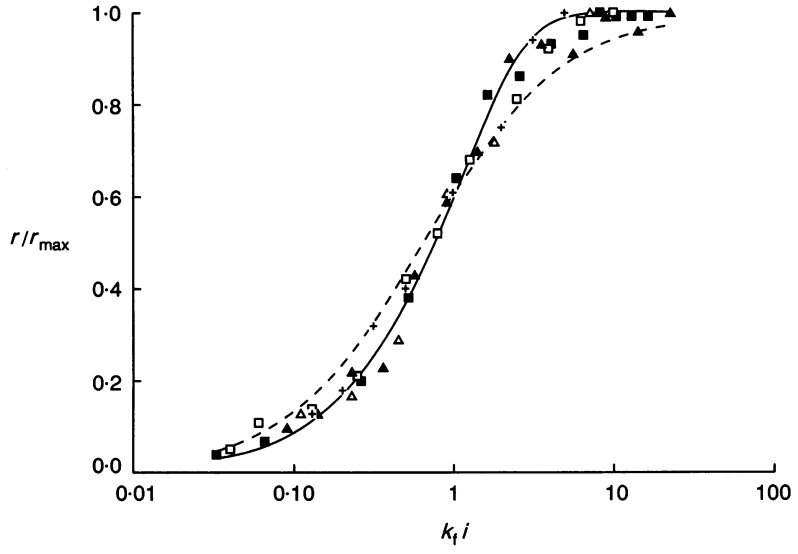

Fig. 2. Normalized response–energy function for five pigeon rods. The continuous curve is the best fit of $r/r_{max} = [1 - \exp(-k_f i)]$; the dashed curve is $r/r_{max} = [i/(i + i_0)]$ (see eqns (3a) and (3b) and following text). The responses of most cells were better fitted by the first function.

Results for six rods with bicarbonate present gave $i_0 = 51 \pm 8$ photons μm^{-2} (mean ± S.D.); in the absence of bicarbonate, however, i_0 exhibited more variability ($i_0 = 72 \pm 51$ photons μm^{-2}, Table 1).

The effective number of photoisomerizations (Rh*) associated with i_0 can be calculated from

$$\text{Rh}^* = i_0 A_c, \tag{4}$$

where A_c, the collecting area, is given by eqn (1). Using the individual values of A_c and i_0 and calculating for each rod, $\text{Rh}^* = 83 \pm 26$ and 133 ± 94 photoisomerizations (means ± S.D.) with and without bicarbonate, respectively.

On the assumption that a length of the outer segment (Δ) is blocked as a result of one photoisomerization, Δ can be estimated if we know k_f from eqn (3a) (Lamb *et al.* 1981). Using eqn (3) in Baylor *et al.* (1984), we have

$$\Delta = \zeta k_f / A_c, \tag{5}$$

where ζ, as before, is the length of the outer segment. As k_f and A_c are known for each cell, we obtain $\Delta = 0{\cdot}27 \pm 0{\cdot}04$ μm (mean ± S.D.) in bicarbonate and $\Delta = 0{\cdot}30 \pm 0{\cdot}26$ μm in the absence of bicarbonate. We report these values of Δ for

the purpose of comparison with other published results; a recent theoretical analysis indicates that the exponential form of the response–energy function does not necessitate the assumption that all of the channels in a defined length of outer segment are inactivated by a single photon (Lamb & Pugh, 1992).

Kinetics of responses to dim flashes

Number of low-pass filter steps

The absorption of a photon by the photoreceptor leads to a chain of molecular events in which the response of the photoreceptor membrane can be simulated by a filter model with n number of first-order steps (Baylor, Hodgkin & Lamb, 1974), although neither n nor the associated time constant(s) have been shown to be identifiable with any of the molecular processes underlying phototransduction. In the Poisson filter model the amplitude of the rod response $r(t)$ is given by

$$r(t) = i\kappa(\alpha t)^{n-1}\, e^{(n-1)(1-\alpha t)}, \tag{6}$$

and in the independent filter model by

$$r(t) = i\kappa e^{-\alpha t}(1 - e^{-\alpha t})^{n-1}, \tag{7}$$

where i is the photon flux, κ is a scaling factor related to the sensitivity of the rod, equivalent to S_{df} (eqn(8)), α is $1/t_{peak}$ and n is the number of low-pass stages (Baylor *et al.* 1979*a*).

Figure 3 shows the response of a rod (heavy curve, rod 7 in Table 1) to a 20 ms dim flash. The thin, smooth curve corresponds to the best fit obtained with eqn (6), for which $n = 4$. (The time between flash and response was corrected for the 20 ms phase delay introduced by the Bessel filter prior to fitting recordings to eqns (6) and (7).) For four cells, best fits were found using the Poisson model, with $n = 4$ in three cases and $n = 5$ in the other. The mean time constant ($\tau = t_{peak}/(n-1)$) was 120 ± 20 ms (mean $\pm$ S.D.). The responses of seven other rods were better fitted by the independent filter model, with $n = 5$ and the five mean values of τ equal to integer multiples of $69 \pm 15{\cdot}7$ ms (mean $\pm$ S.D.) (Table 1, upper data set). In some cases modelling the rod response to fit the falling phase of the photocurrent was not possible with either the Poisson or independent stage model (Fig. 3). In the presence of bicarbonate the two models provided equally good fits ($n = 4$ (or 5 in one case) and $\tau = 105 \pm 39$ ms with the independent filter ; $n = 3$ (or 4) with the Poisson model).

Time to peak and integration time

For dim flashes the time to peak (t_{peak}) was 315 ± 73 and 327 ± 107 ms (mean $\pm$ S.D.), without and with bicarbonate respectively, as measured from the middle of a 20 ms light pulse. The integration time was 572 ± 126 ms (means $\pm$ S.D.) in the absence of bicarbonate but increased to 851 ± 86 ms when bicarbonate was present.

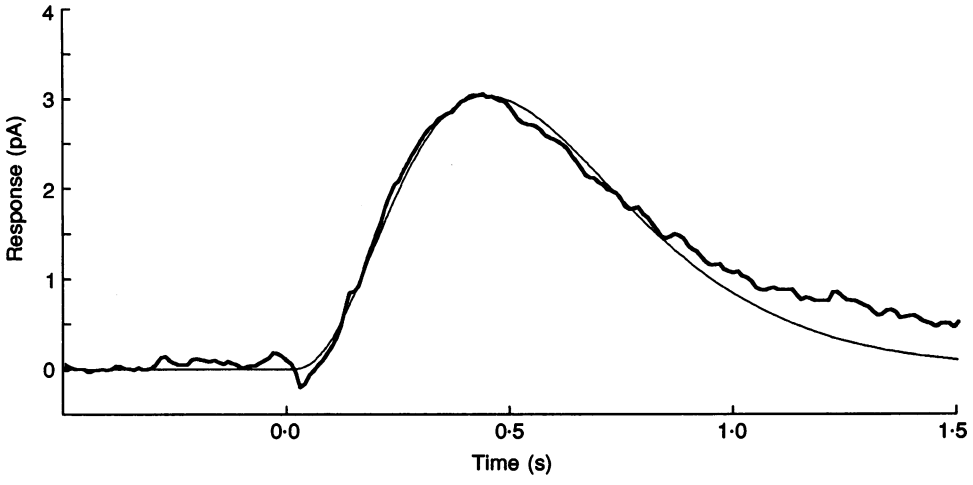

Fig. 3. Heavy curve: response of a rod to 20 ms dim flashes of light (average of 89 stimuli). Flash intensity was 10 photons μm^{-2}. Light curve: calculated response based on the Poisson model (eqn (6)) with four filter stages ($n = 4$).

Flash sensitivity

The rod sensitivity to a dim flash is defined by

$$S_{df} = r(t)_{peak}/i, \tag{8}$$

where $r(t)_{peak}$ is the peak amplitude of the rod response in the range of intensities where the response increases linearly, and i is the intensity in photons per square micrometre. Rod sensitivity was $0{\cdot}56 \pm 0{\cdot}19$ pA photon^{-1} μm^2 (mean $\pm$ S.D.) in bicarbonate and $0{\cdot}22 \pm 0{\cdot}16$ μm^2 without bicarbonate.

The amplitude of the response to single photons (S_ϕ) can be estimated from S_{df}:

$$S_\phi = S_{df}/A_c. \tag{9a}$$

$S_\phi = 0{\cdot}37 \pm 0{\cdot}19$ pA photon^{-1} (mean $\pm$ S.D.) in bicarbonate and $0{\cdot}13 \pm 0{\cdot}13$ in the absence of bicarbonate. Expressed as the percentage of the light-modulated conductance suppressed per photon, these values correspond to $1{\cdot}21 \pm 0{\cdot}47$ % and $0{\cdot}84 \pm 0{\cdot}68$ %, respectively (Table 1).

An alternative way to estimate S_ϕ without measuring either A_c or calibrating the light source comes from an analysis of variance of the Poisson-distributed responses of rods to dim flashes, using the logic employed by Katz & Miledi (1972) to determine the amplitude of quantal events involved in synaptic transmission:

$$S_\phi = \sigma^2/\mu, \tag{9b}$$

where μ is the mean peak amplitude of a series of events, and σ^2 is the variance at the peak for this same series. Using this approach, $S_\phi = 0{\cdot}31 \pm 0{\cdot}11$ pA per

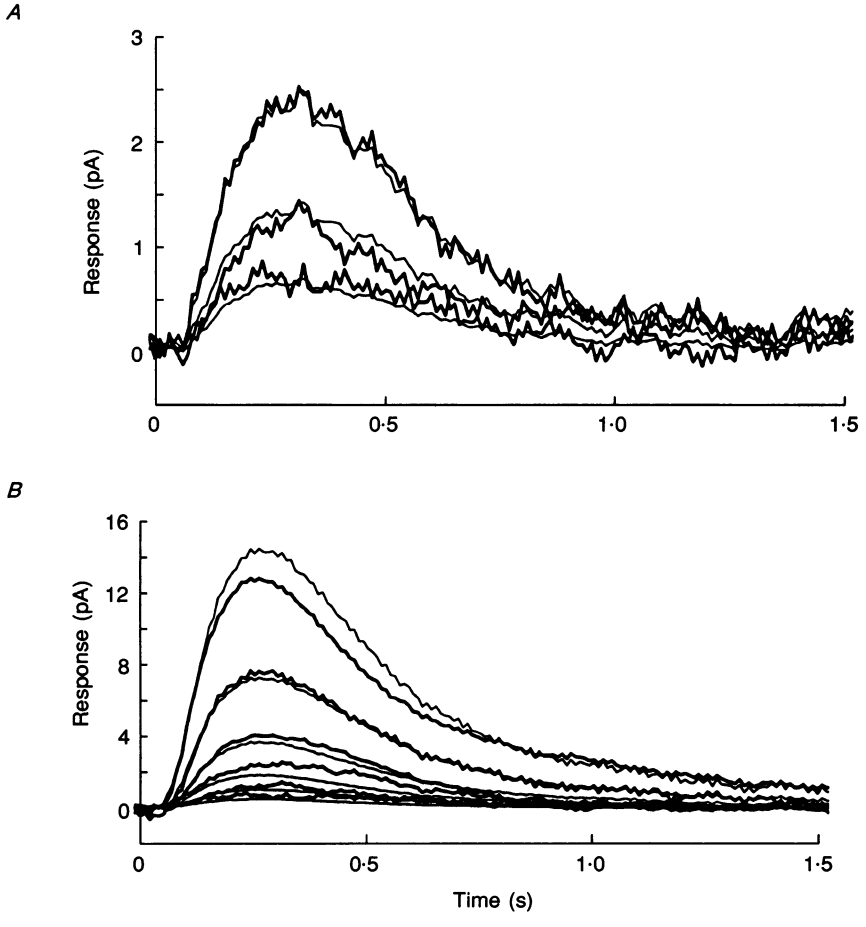

Fig. 4. *A*, heavy curves: responses of a rod to 20 ms flashes of 8·5, 17 and 30 photons μm^{-2} (each curve is an average of four to six responses). Light curves: appropriately scaled template formed by normalizing and averaging the three responses. Agreement of responses and template indicates that for small responses the photocurrent increases linearly with intensity through the entire course of the response. *B*, similar to *A*, except six flash intensities were used in generating the template, the highest being 240 photons μm^{-2}. At several intensities there are differences between the response and the template, but these are not present early in the response. This result suggests that for all but the dimmest flashes there is detectable non-linearity at times greater than 150 ms.

photoisomerization (mean ± S.D.) for seven cells that were measured in the absence of bicarbonate. Expressed as the percentage of current that is blocked by light, this is 2·2 ± 0·84 % (mean ± S.D.).

Linearity of responses to flashes and steps

Over what range are the responses of pigeon rods linear with intensity? This question was approached by averaging responses of a cell to flashes at several intensities to generate an empirical 'template'. Responses of the cell at each intensity i were then compared with the template, after scaling the latter by i/i_{av},

where i_{av} is the average intensity of the flashes used in generating the template. To the extent that responses are linear with intensity, they should be fitted by the template (Baylor & Hodgkin, 1973).

The heavy curves in Fig. 4*A* are responses of a rod to 20 ms flashes of 8·5, 17 and

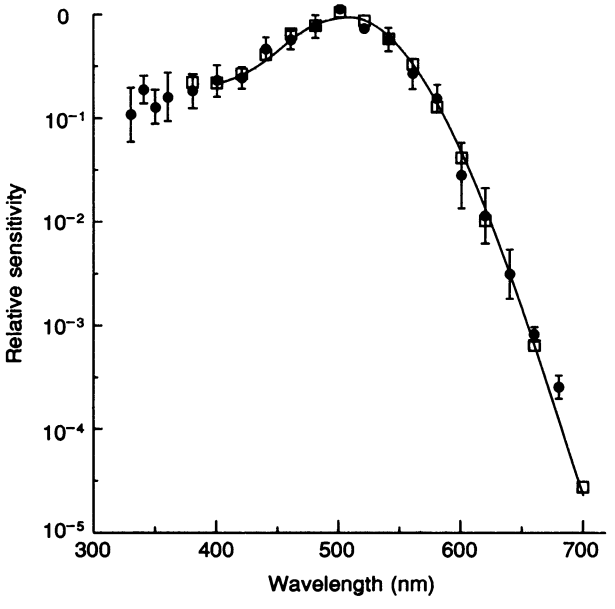

Fig. 5. Spectral sensitivity of pigeon rods compared with behavioural measurements of scotopic sensitivity and of absorbance of rhodopsin in digitonin extracts. ●, currents from single rods ± S.D. ($n = 17$); present work. □, scotopic sensitivity function, key-pecking procedure (Blough, 1957), quantized and corrected for transmission of the ocular media (see text). Curve, generalized rhodopsin spectrum with λ_{max} at 505 nm, based on a polynomial function devised by Gary D. Bernard (unpublished). The λ_{max} of sensitivity for individual rods was between 497 and 508 nm. There is a low shoulder of sensitivity extending to 330 nm in the near-UV, the effective short-wavelength limit of our apparatus.

30 photons μm^{-2}. (Each curve is the mean of four to six responses.) The thin curves describe the appropriately scaled template, which was formed by averaging the three heavy curves. The agreement of curves and template indicates that for small responses (0·15 peak amplitude in this cell), the photocurrent is linear with flash intensity. Figure 4*B* is similar, except six flash intensities were used in generating the template, the highest being 240 photons μm^{-2}. At several intensities there are differences between the response and the template, but these are not present early in the response. This result suggests that for all but the dimmest flashes there is detectable non-linearity at times greater than 150 ms. For five cells that were analysed in this fashion, non-linearity was observed when more than 24 % (range 13–32 %) of the light-modulated conductance was suppressed.

Spectral sensitivity

Spectral sensitivity was determined between 330 and 680 nm, using the procedure described in the Methods. Figure 5 (●) shows the mean spectral

sensitivity (± S.D.) of seventeen rods. The curve is a polynomial function devised by G. D. Bernard (unpublished) which describes well the absorption of vertebrate rhodopsins; the best least-squares fit to the data at $\lambda > 400$ nm was achieved with $\lambda_{max} = 505$ nm. There is a low shoulder of sensitivity extending to 330 nm in the near-ultraviolet wavelengths (near-UV), the effective short-wavelength limit of our apparatus. The open squares show the spectral sensitivity of scotopic vision in the pigeon, determined by a behavioural method (Blough, 1957), and shifted vertically for the best fit to the same polynomial function.

DISCUSSION

These results on pigeon rods represent the first detailed study on the photocurrents of photoreceptors from the other non-mammalian group of warm-blooded animals, birds. Unlike mammals, the pigeon has a predominantly diurnal visual system in which cones outnumber rods by about 2:1 over much of the retina, and in specialized regions rods may account for only 10–20 % of the receptors (Schultze, 1866). Comparison with previous results on mammalian rods are therefore interesting.

The rods of pigeons appear to generate somewhat smaller responses to single photons than do the rods of many mammals. S_ϕ was found to be 0·37–0·43 pA photon^{-1} in the present study, whereas corresponding values for mammalian rods are 0·32–0·97 pA photon^{-1} in primates (Baylor *et al.* 1984; Tamura, Nakatani & Yau, 1991), 0·54–0·81 in rat, cattle and rabbit (Nakatani *et al.* 1991), and 1·12 pA photon^{-1} for the cat (Tamura *et al.* 1989). Expressed as the percentage of the light-modulated conductance shut down by a single photon, these figures correspond to about 1·2 % for pigeon (0·84 % in the absence of bicarbonate) and 2·7–6·2 % or more for primates and other mammals. The largest values seem to occur in the rods of nocturnal species. Similarly Δ, originally interpreted as the length of the outer segment over which sodium conductance is shut down by a single activated rhodopsin, is 0·27–0·30 in pigeon rods but 0·52–1·07 in various mammals.

Non-linearity of the photocurrent of pigeon rods is first evident in the recovery phase of the response. In the five cells examined in detail, non-linearity set in when there had been about ten photoisomerizations.

The addition of bicarbonate ions to the recording solution led to somewhat larger responses and less variability in i_0. The time to peak was not altered, but the responses were slower to decay when bicarbonate was present. The closest comparison with mammals is with the monkey *Macaca fascicularis* (Baylor *et al.* 1984), where in the absence and presence of bicarbonate the time to peak was 270 and 190 ms respectively (cf. 315 and 327 ms for pigeon), while the integration time decreased from 330 to 280 ms (cf. 572 and 851 ms for the pigeon). The effect of bicarbonate on the integration time is thus qualitatively different in pigeon and monkey, and the responses of pigeon rods are slower than those of primates at the same temperature.

The spectral sensitivity function of pigeon rods fits a rhodopsin template with λ_{max} at 505 nm and is in good agreement with behavioural measurements of scotopic sensitivity of the pigeon (Blough, 1957). Blough's data in Fig. 5 have been converted from energy units to relative numbers of photons and further corrected for the transmission of the ocular media of the pigeon, using the measurements of

Emmerton, Schwemer, Muth & Schlecht (1980). Blough's spectral sensitivity curve shows no evidence of the self-screening that should be present *in vivo* (the curve is no broader than the rhodopsin template), and when adjusted for estimated axial absorbance, the fit to the template deteriorates slightly. As plotted in Fig. 5, his data have therefore not been corrected for self-screening. Bridges (1962) measured the spectral absorbance of pigeon rhodopsin in detergent solution; his spectrum has a very similar bandwidth to the spectra in Fig. 5, but the λ_{max} is at 500 nm. The absorption spectra of individual pigeon rods made by microspectrophotometry (Bowmaker, 1977) have λ_{max} at 503 nm but are somewhat broader than the spectra in Fig. 5.

Because the lens and ocular media of birds are transparent through much of the near-UV (Emmerton, Schwemer, Muth & Schlecht, 1980; Goldsmith, 1990) and many birds are known to have a cone with maximum sensitivity at about 370 nm (Chen & Goldsmith, 1986), we have measured spectral sensitivity down to 330 nm, further into the near-UV than has been customary in similar studies of vertebrate photoreceptors. Furthermore, because of light scatter, low lamp energy, and the presence of photoproducts, this is a difficult region of the spectrum in which to obtain estimates of spectral sensitivity by microspectrophotometric measurements of absorption. The sensitivity of pigeon rods does not show evidence of a pronounced β-band of absorption in this spectral region, although the long-wavelength-sensitive cones of pigeons do (A. G. Palacios & T. H. Goldsmith, in preparation).

This research was supported in part by grants EY-00222 and EY-00785 to Yale University from the National Eye Institute, National Institutes of Health. A.G.P. was the recipient of fellowship support from the Simone & Cino del Duca and Philippe, Inc. Foundations. John Douglass participated in the early phases of the project and was involved in the development of the software and efforts to define optimal recording conditions for avian photoreceptors. We thank Gary D. Bernard for the use of his unpublished polynomial equation that describes the absorbance spectra of vertebrate rod pigments and Denis Baylor for reading and commenting on the penultimate draft of the manuscript.

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