The Response to Monochromatic Light Flashes of the Oxygen Consumption of Honeybee Drone Photoreceptors

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ABSTRACT   Local measurements of the fall in oxygen pressure on stimulation of slices of the retina of the honeybee drone by flashes of light were made with oxygen microelectrodes and used to calculate the kinetics of the extra oxygen consumption (ΔQ_{O_2}) induced by each flash. The action spectrum for ΔQ_{O_2} was obtained from response-intensity curves in response to brief (40 ms) monochromatic light flashes. The action spectrum of receptor potentials was obtained with the same experimental conditions. The two action spectra match closely: they deviate slightly from the photosensitivity spectrum of the drone rhodopsin (R). The deviation is thought to be due to wavelength-dependent light scattering and absorption in the preparation. In these experiments, the visual pigment was first illuminated with orange light, which is known to convert the bistable drone photopigment predominantly to the R state from the metarhodopsin (M) state. When long (300–900 ms) light flashes were used to elicit ΔQ_{O_2}, the responses to different wavelengths could not be matched in time course (as for the short flashes). Flashes producing large R-to-M conversions produced a prolonged ΔQ_{O_2}. The prolongation did not occur after double flashes, which produced both large R-to-M and M-to-R conversions. Similar changes in the length of afterpotentials in the photoreceptor cells and in a long-lasting decrease in photoreceptor intracellular K^+ activity were found after long single or double flashes. The results are interpreted to show that the initial event for stimulation by light of metabolism in the drone retina is the same as that for stimulation of electrical responses (i.e., absorption of photons by R). Absorption of photons by M can produce an inhibitory effect on this stimulation.

INTRODUCTION

In the honeybee drone retina, only the sensory neurons (the photoreceptors) contain significant numbers of mitochondria. Because of this feature and because it is possible to stimulate these regularly arranged neurons homogeneously with a very brief light stimulus, it is an excellent preparation of nervous tissue in which to study the regulation of oxidative metabolism. This was indeed the first

nervous tissue for which the complete time course of O_2 consumption was measured: in a superfused slice of retina, a single flash of white light produces a transient increase in oxygen consumption (ΔQ_{O_2}), with a peak occurring after 2–3 s and a return to resting level after ~12 s (Tsacopoulos and Poitry, 1982). The extra metabolic energy produced by this ΔQ_{O_2} is presumably necessary to restore the photoreceptor (and glial) cells to their resting, dark-adapted state. For example, it is estimated (Tsacopoulos et al., 1983) that about half of the extra metabolic energy produced is consumed by the Na pumping needed to extrude the extra Na ions that accumulate (Coles and Orkand, 1982) within the photoreceptors because of the increased influx triggered by the light stimulus. However, in contrast to an earlier hypothesis (Tsacopoulos and Poitry, 1982), the rise of ΔQ_{O_2} occurs before the extrusion of Na ions, which implies that it is not the increase in the Na pumping rate that is the stimulus for the increase in the metabolic rate (Tsacopoulos et al., 1983). In the present work, therefore, we have addressed the problem of whether the initial stimulation of metabolism represented by ΔQ_{O_2} is the absorption of light by rhodopsin. If so, then the action spectrum of ΔQ_{O_2} should correspond with the photosensitivity spectrum of rhodopsin.

Microspectrophotometry of single rhabdoms of the drone retina has shown that the major visual pigment is a bistable pigment with a rhodopsin (R) state absorbing in the violet and a metarhodopsin (M) state absorbing in the green (Bertrand et al., 1979; Muri and Jones, 1983). Stimulation with white light (after white-light adaptation) therefore transfers pigment molecules between the two states, but does not produce any change in the amount of R and M (see Hillman et al., 1983). Here we describe measurements of ΔQ_{O_2} after stimulation with colored light. With this condition, the effect of the light stimulus on the photopigment depends both on the wavelength of the stimulus and on the previously adapted state of the photopigment. The amount of phototransformation can be calculated from a knowledge of the absorption spectra of the R and M states and of the wavelength, intensity, and duration of the stimulus. The equations for such calculations are developed below in the Appendix.

We measured the action spectrum of ΔQ_{O_2} in slices of the drone retina using short monochromatic light flashes that were calculated to convert only a small fraction of the photopigment from R to M. When longer monochromatic light flashes were used as the stimulus, a wavelength-dependent change in the kinetics of ΔQ_{O_2} was found. In the second part of this article, we describe this effect: a prolongation of the time course of ΔQ_{O_2} after a flash. It appears to be due to a relatively large net photoconversion of R to M, and is not present if there also is a reconversion of M to R.

Part of this work has previously published in the form of an abstract (Jones and Tsacopoulos, 1985).

METHODS

Experimental Procedures

The experiments described here were done using 300-μm slices of the honeybee drone retina, obtained, after decapitation, by making two parallel cuts with a vibrating razor

blade, parallel to the ommatidia in the dorsal region of the retina. The slices were mounted in a perfusion chamber, which provided laminar flow of the oxygenated physiological solution over one surface of the preparation. The other surface lay flush with a flat plate of either glass or stainless steel (Tsacopoulos et al., 1981; Tsacopoulos and Poitry, 1982). The physiological solution contained (mM): 283 NaCl, 10 KCl, 1.6 $CaCl_2$, 5 $MgCl_2$, 10 Tris-HCl, pH 7.4, at 22°C. The perfusion chamber was situated inside a light-tight cage, in which the slices were allowed to dark-adapt for at least 30 min before each experiment.

Light stimulation was by means of one or two collimated light beams from 150-W xenon lamps (XBO 150, Osram GmbH, Munich, Federal Republic Germany [FRG]). The beams were passed through neutral density and interference filters, or a cut-on filter, heat filters, and electronic shutters, before being combined and then focused on the surface of the retinal slices with a microscope objective.

Calibration of the Light Stimuli

The relative incident photon flux with each of the different interference filters in place was measured using a photodiode (OSO 50-1, Centronic Ltd., Croydon, U.K.) placed in the same position as the preparation. The light intensities were taken to be proportional to the voltage output, after current-to-voltage conversion, and after correction for the wavelength dependence of the quantum efficiency of the photodiode. The quantum efficiency was found to be flat between wavelengths of 460 and 600 nm by comparison with a calibrated photometer (J16/J6502, Tektronix Inc., Beaverton, OR). The quantum efficiency fell at wavelengths below 450 nm; this was measured by comparison with a second, similar photodiode, which was specially calibrated between 350 and 470 nm by Centronic Ltd.

The incident photon flux with the 410-nm interference filter was measured as 1.5 × 10^{15} photons cm^{-2} s^{-1} using a photomultiplier that had been calibrated absolutely at Garching, Munich, FRG. Unattenuated light intensities at other wavelengths increased from −0.57 (at 396 nm) to +0.77 log units (at 527 nm), as measured with the photodiode. The relative intensities given in the figures and figure legends are relative to the maximum monochromatic intensity available, which was therefore 8.8 × 10^{15} photons cm^{-2} s^{-1}, at 527 nm.

The transmission spectra of the interference filters (obtained from Balzers AG, Lichtenstein), neutral density filters, and heat filters were measured using a Cary recording spectrophotometer (Varian Associates Inc., Palo Alto, CA). The wavelengths quoted for each interference filter are those for which transmission was maximal, with one exception. The passband of the filter nominally maximal at 383 nm was found to overlap with the low-wavelength cut-off of the heat-absorbing filter. The combination of the two filters produced a flatter passband with a midpoint at ~396 nm and a width of 14 nm at 50% of maximum. The bandwidth of the other interference filters was 6 or 7 nm at 50% of maximum.

The transmission of the neutral density filters was flat to within 5% for wavelengths from 370 to 600 nm. Errors caused by reflections when combinations of filters were used in the light beams were measured using the photodiode and found to be <5% when compared with the expected transmission from individual transmission measurements.

Adaptation and Stimulation with Colored Light

It is not possible, at present, to measure either absorption of light by the visual pigment or the photoconversion of the pigment in retinal slices, because the absorbance owing to the pigment is only a small fraction of the total absorbance of the slices. Indeed, during measurements of the transmission of retinal slices, we were unable to detect any change

in transmission caused by phototransformation from R to M or M to R during alternating prolonged violet or green illumination. (Given the noise level of the photodiode, a change of ~5% in absorbance should have been detectable.) However, the effect of light stimuli on the occupancy of R and M states of the pigment was estimated as follows. Fig. 1 shows the photosensitivity spectra of the R and M states of the major visual pigment of the dorsal part of the drone retina, the part used in the present experiments. These spectra were obtained from the original data of Muri and Jones (1983) from microspectrophotometry of single rhabdoms, after recalculation using a relative quantum efficiency for phototransformation between R and M of 0.71 (Cronin and Goldsmith, 1982) rather than unity, and have been scaled so that the absorption coefficient at the R peak corresponds to a molar extinction coefficient of 40,000 liter mol^{-1} cm^{-1}, as measured for crayfish rhodopsin (Larrivee and Goldsmith, 1982). This peak extinction is close to that measured
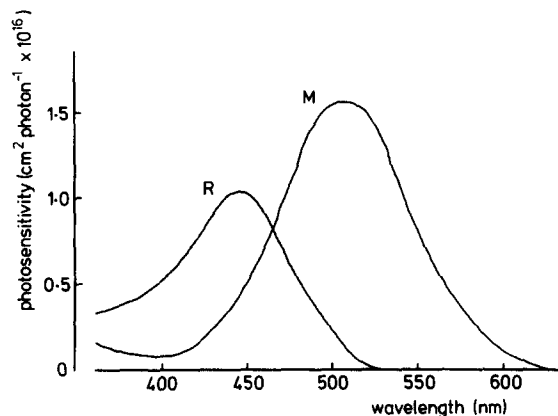

FIGURE 1. Photosensitivity spectra of the rhodopsin (R) and metarhodopsin (M) states of the major photopigment in the dorsal region of the drone retina. These spectra were recalculated from the original data used for obtaining the relative absorption spectra of R and M (see Muri and Jones, 1983), with the additional information that the relative quantum efficiency for R-to-M photoconversion is 0.71 (Cronin and Goldsmith, 1982). The two spectra are scaled so that the absorption peak of R corresponds to a molar extinction of 40,000 liter mol^{-1} cm^{-1}.

for many different rhodopsins, both in extracts and in situ (Dartnall, 1972; Hillman et al., 1983). The major change from the previously published absorption spectra of the drone R and M (Muri and Jones, 1983) (apart from the different relative heights of the photosensitivity spectra), which arises from the use of a different value for the relative quantum efficiency, is a sharpening of the M spectrum on the low-wavelength side of its peak.

From Fig. 1 it is clear that continuous violet light of wavelength 410–420 nm is most effective in converting the drone R to M. This produces a steady state mixture with ~86% M and 14% R. Orange light, of wavelengths >530 nm, converts M to R to a steady state with >99% R (Muri and Jones, 1983; and see Appendix). The time constants of the approach to these steady states (which are independent of the starting conditions; Hochstein et al., 1978) are estimated (see Appendix) to be 9 and 3 s under our experimental conditions. Adaptation with orange light (using a 530-nm cut-on filter) was therefore done for a time of at least 30 s, to produce >99% R in the retinal slices, and adaptation

with violet light was for a period of 2 min, producing 14% R, 86% M. The preparations were subsequently dark-adapted for at least 15 min.

As described in the Appendix, an estimate can be made of the fraction of photopigment converted from R to M or M to R by different colored-light flashes. Table I shows the calculated values of the fractional photoconversion of pigment molecules by various stimuli used in the present study. The first two lines show the typical photoconversion by short, 40-ms stimuli. These are estimated to convert <2% of the pigment from R to M with negligible reconversion, and were used in the first part of this study to obtain the action spectrum for ΔQ_{O_2}.

Table I shows that much more significant photoconversion occurred for longer flashes, but the amount depended very much on the stimulating wavelength and the initial conditions. A single long violet flash and a single long green flash are predicted to convert ~6% and ~0.5% R to M, respectively, the latter amount being similar to the photocon-

TABLE I

Photoconversion of Rhodopsin to Metarhodopsin by Different Light Stimuli*

Light stimulus	Initial state	R → M	M → R	Net R → M
		%	%	%
Violet, 410 nm, 40 ms	100% R	0.4	10^{-4}	0.4
Blue, 450 nm, 40 ms	"	1.9	10^{-4}	1.9
Violet, 410 nm, 600 ms	"	5.5	3×10^{-2}	5.5
Green, 500 nm, 600 ms	"	11	4	7
Green, 527 nm, 600 ms	"	0.7	0.3	0.4
Violet, 410 nm, + green, 500 nm, 600 ms	"	16	5	11
Violet, 410 nm, + green, 527 nm, 600 ms	"	6	2	4
Violet, 410 nm, 600 ms	86% M (violet adapted)	0.8	0.8	0
Violet, 410 nm, + green, 527 nm, 600 ms	"	3	55	−52
White, 40 ms	60% R	40	40	0

* The values are for unattenuated light stimuli and are expressed as percent of the total pigment present. They were calculated using Eqs. 5 and 6 of the Appendix.

version caused by a short flash. In comparing responses to a long violet flash with those to a combined violet and green flash (see Figs. 9 and 10), the double flash is predicted to produce about the same or a larger R-to-M conversion, but with a significant M-to-R reconversion, which did not occur for the single flash. Finally, for a violet-adapted preparation, in comparison with an orange-adapted preparation, the amount of R-to-M photoconversion was small for a violet flash; it was larger for a combined violet and green flash, but was then accompanied by a very large M-to-R conversion.

The last line of Table I gives, for comparison, an estimate of the amount of conversion by the (unattenuated) white-light stimuli used in the previous study of the light-stimulated O_2 consumption of the drone retina (Tsacopoulos and Poitry, 1982).

Measurement of ΔQ_{O_2}

Two double-barreled, recess-tipped O_2 microelectrodes (with tip diameters of <3 μm and a recess length of ~10 μm) were used to follow changes in tissue P_{O_2} after light flashes. One of these was placed at the surface of the retina by visual control. The second was placed at a known depth in the retinal slice (100–200 μm) using a micromanipulator

connected to a calibrated multi-turn potentiometer. The current outputs from the electrodes were converted to voltages, amplified, digitized, and stored on magnetic disks. The extra O_2 consumption above the basal, dark level as a function of time after the flash was calculated from these records by solving the equation linking O_2 consumption with O_2 diffusion by means of a discrete fast Fourier transform routine, using previously determined values for the solubility and diffusion coefficients of O_2 in the drone retina (Tsacopoulos et al., 1981). The electrodes were calibrated by measuring their responses in O_2-saturated and air-saturated physiological solution. Full details and a discussion of the recording system and the calculations are given elsewhere (Tsacopoulos and Poitry, 1982).

Electrical Recordings

Intracellular receptor potentials from the photoreceptor cells were measured using conventional glass microelectrodes (resistances ~30 MΩ) and conventional recording techniques. The photoreceptor cells recorded from had resting potentials >50 mV, and were situated in the central regions of the retinal slices, at depths between 100 and 200 μm. Measurements of changes in $[K^+]$ in the photoreceptor cells after a flash were made using double-barreled K^+-sensitive microelectrodes constructed as described elsewhere (Coles and Tsacopoulos, 1977; Munoz et al., 1983).

RESULTS

ΔQ_{O_2} after Short Monochromatic Light Flashes

Fig. 2A shows the ΔQ_{O_2} after 40-ms flashes of wavelengths 396, 430, and 450 nm in the same slice. For short, 40-ms light flashes, the ΔQ_{O_2} was found to be similar to that after white-light flashes, though of lower amplitude because of the weaker light intensities. The ΔQ_{O_2} rose to a peak after 2–3 s and then returned to the baseline after 10–15 s. The responses at different wavelengths had the same form, as shown in Fig. 2B, where three responses, from a different slice, have been matched at their peak amplitude. We found that, although there were differences between slices, in the same slice the form of the response to different monochromatic light flashes was independent of wavelength. Moreover, the form of the ΔQ_{O_2} responses was also independent of intensity at any given wavelength, as found previously for white-light flashes (Tsacopoulos and Poitry, 1982).

For white-light flashes, either the amplitude of the ΔQ_{O_2} or the integral, the total ΔQ_{O_2} induced by the light flash, increases linearly with the logarithm of the light intensity (Tsacopoulos and Poitry, 1982). Fig. 3 shows the total ΔQ_{O_2} in one preparation after monochromatic light flashes of different intensities at four different wavelengths. Except for very low-level responses, the total ΔQ_{O_2} increases linearly with the logarithm of light intensity, and the slope of the relation between the two is the same for the different wavelengths. Although the slope varied somewhat between slices, a similar result was found for stimulation at two or more wavelengths in five different slices. A "tail-off " at low light intensities (see Fig. 3, at 527 nm) could usually be detected just above noise levels, which indicates that the response tends to linearity at very low light levels, as expected. There was no indication of saturation of the ΔQ_{O_2} responses at the highest light intensities available, even though these were just sufficient to saturate the electrical responses of the photoreceptor cells (see below). A similar discrepancy
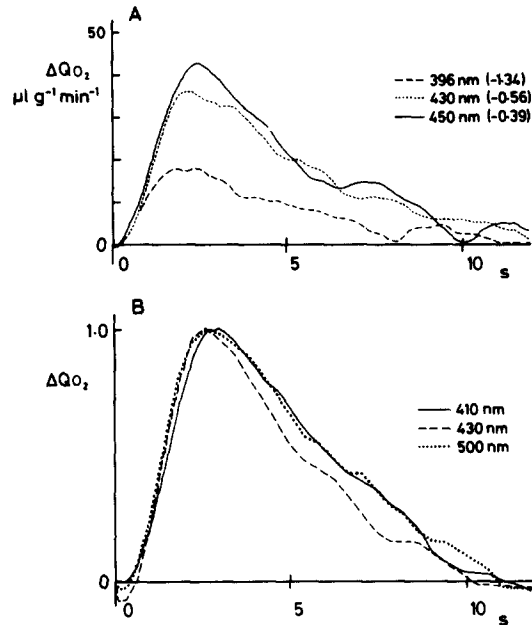
FIGURE 2.   Transient increases in O_2 consumption (ΔQ_{O_2}) in slices of honeybee drone retina after monochromatic light flashes. (A) Superimposed tracings of ΔQ_{O_2} after flashes of wavelengths 396, 430, and 450 nm. Flash duration, 40 ms. Numbers in parentheses are log relative light intensities for the three flashes (see Methods). (B) From a different experiment, superimposed tracings of ΔQ_{O_2} induced by monochromatic light flashes after normalization at the peak amplitude, showing that the kinetics of the response are independent of the wavelength of the stimulating light. Flash duration, 40 ms.
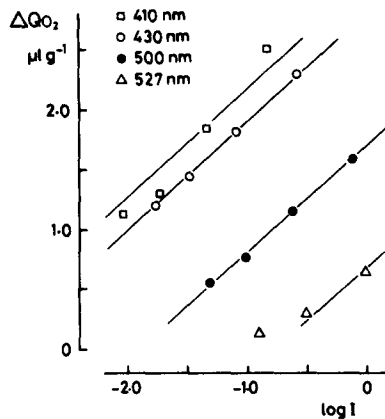

FIGURE 3.   Amplitude of the total ΔQ_{O_2} in a slice (calculated as the integrated ΔQ_{O_2}) after 40-ms flashes of monochromatic light, as a function of the light intensity (I) at different wavelengths. The lines have the same slope and were drawn by eye through the data points. The slopes of the (total ΔQ_{O_2})/(log I) lines varied from one slice to another, but in any one experiment were constant over the wavelength and intensity ranges tested. Note the tail-off of ΔQ_{O_2} at low light intensities (see text).

was previously described for stimulation by white-light flashes (Tsacopoulos and Poitry, 1982).

The above findings, that the ΔQ_{O_2} responses of different wavelengths may be matched in shape and have a similar dependence on light intensity, show that the principle of univariance holds (Naka and Rushton, 1966) and make it likely that the initial stimulus for the responses is absorption by a single photopigment. The action spectrum for ΔQ_{O_2} was obtained from a series of experiments in which at least one complete response/light intensity line was measured for each slice. Using the slope of this line, extrapolation from the responses of other wavelengths was used to calculate the light intensity for a criterion response of a total ΔQ_{O_2} of 1 μl g^{-1}, about the midpoint of the range over which measured responses were linear with log light intensity. At the peak sensitivity, the light
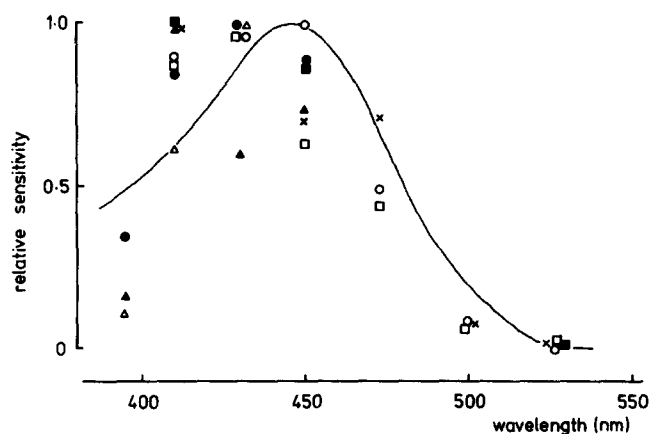

FIGURE 4. Action spectrum of ΔQ_{O_2} after monochromatic light flashes. Each symbol represents results from a different experiment. The curve is the normalized photosensitivity spectrum of the drone rhodopsin (see Fig. 1).

intensity for the criterion response was $\sim 10^{14}$ photons cm^{-2} s^{-1}. The relative sensitivities at different wavelengths were calculated as the inverse of the light intensity for the criterion response and normalized to the maximum value (Naka and Rushton, 1966; Tomita et al., 1967; Baylor and Hodgkin, 1973). This procedure was adopted since the preparations were not stable for a time sufficient to obtain a complete series of response/light intensity curves at all wavelengths.

Fig. 4 shows the results of the individual experiments designed to measure the action spectrum of ΔQ_{O_2}. The points are the relative sensitivities calculated from the total ΔQ_{O_2} after monochromatic light flashes as described above. In Fig. 7, the same results are shown after averaging and are plotted on a logarithmic rather than a linear scale. In both figures, the photosensitivity spectrum of the drone R, as determined from optical density difference measurements on single rhabdoms (see Fig. 1), has also been plotted.

The action spectrum of ΔQ_{O_2} is similar to that of the R absorption spectrum, but it does not correspond exactly. The fall-off toward the green occurs at

shorter wavelengths than for the absorption spectrum, and the peak also appears at shorter wavelengths.

Action Spectrum of Electrical Responses

Previous measurements of the action spectrum of receptor potentials in drone photoreceptor cells (Bertrand et al., 1979) used very weak light stimuli directed through the dioptric apparatus and, therefore, along the rhabdoms of the cells. In that situation, the action spectrum of the responses corresponded quite well
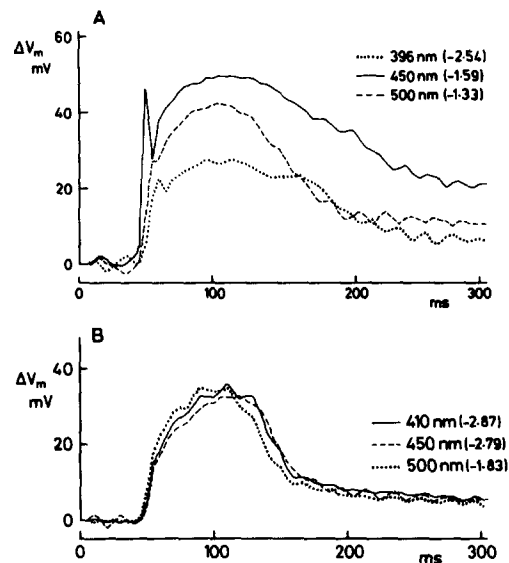

FIGURE 5.   Electrical responses of a drone photoreceptor cell recorded under the same conditions as the experiments measuring ΔQ_{O_2}. (A) Superimposed tracings of receptor potentials after monochromatic light flashes. (B) From the same experiment, superimposed tracings of receptor potentials of similar amplitude after different monochromatic light flashes, showing that the form of the response is independent of the wavelength of the stimulating light. Flash duration, 40 ms. Resting membrane potential, 55 mV. The numbers in parentheses are log relative light intensities for the different flashes. The initial spike and the rising phase of these electrical responses were partly clipped by the recording electronics during analog-to-digital conversion.

with the R absorption spectrum. For the present work, there were two differences in experimental conditions: the light stimuli were much stronger (to obtain measurable values of ΔQ_{O_2}), and stimulation was transversal to the cells, rather than along the rhabdoms. It might be that the discrepancy between the action spectrum of ΔQ_{O_2} and the R absorption spectrum (Figs. 4 and 7) is due to the particular experimental arrangements. We therefore measured the action spectrum of electrical responses with the present experimental arrangements.

When stimulated with short flashes of white light, the receptor potentials of the drone photoreceptor cells show changes in waveform that are dependent on

light intensity (Baumann, 1968). The same holds for stimulation with monochromatic light flashes (Fig. 5A). However, we found that by varying the intensity, the receptor potentials for different monochromatic flashes could be closely matched in form when the responses were similar in amplitude (Fig. 5B). Moreover, the peak amplitude of the receptor potentials after monochromatic light flashes was found to be linearly related to the logarithm of the light intensity (Fig. 6), as for white-light flashes of similar intensity (Baumann, 1968). At peak sensitivity, the linear relationship held, in the present study, for light intensities that were −2 to about −4 log units less than reference (see Fig. 6), i.e., between $\sim 10^{14}$ and 10^{12} photons cm^{-2} s^{-1}. This range therefore overlaps with the range
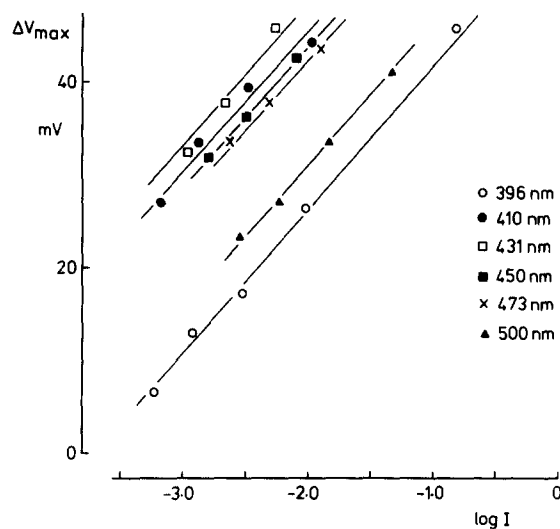

FIGURE 6. Peak amplitude of receptor potentials after monochromatic light flashes as a function of light intensity (I) at different wavelengths. Parallel straight lines have been drawn by eye through the experimental points. Same experiment as in Fig. 5.

in which ΔQ_{O_2} responses are linear with log light intensity (from $\sim 10^{13}$ to $>10^{15}$ photons cm^{-2} s^{-1}). At higher intensities, the electrical responses saturated; this detail has been omitted from Fig. 6. The action spectrum for the receptor potentials was obtained from a series of experiments that provided graphs such as those shown in Fig. 6. The action spectrum was obtained by reading off the light intensity for a response amplitude of 27 mV at each wavelength, and then plotting the inverse of the light intensity, the relative sensitivity, against the wavelength of the light.

The averaged results (Fig. 7) indicate an action spectrum for the receptor potentials that is very similar to the action spectrum for ΔQ_{O_2}. Within experimental error, the two are indistinguishable.

ΔQ_{O_2} after Long Colored-Light Flashes

As described above, the ΔQ_{O_2} responses after short light flashes had the same form, independent of intensity and wavelength even at the highest light intensities

available with the interference filters in place. When slices were stimulated with longer unattenuated light flashes, this was found no longer to hold. The wavelength-dependent change in the kinetics of the responses was a change in the falling phase only, and this occurred for stimulation at wavelengths close to 410 nm, where the slices were most sensitive. When stimulation was with long unattenuated flashes at these shorter wavelengths, the rising phase of ΔQ_{O_2} was similar to the rising phase of ΔQ_{O_2} at longer wavelengths, but the falling phase became prolonged; i.e., the ΔQ_{O_2} returned more slowly to the baseline. This effect was variable in extent from one preparation to another, but was always present when looked for, even though in some cases the change in kinetics was
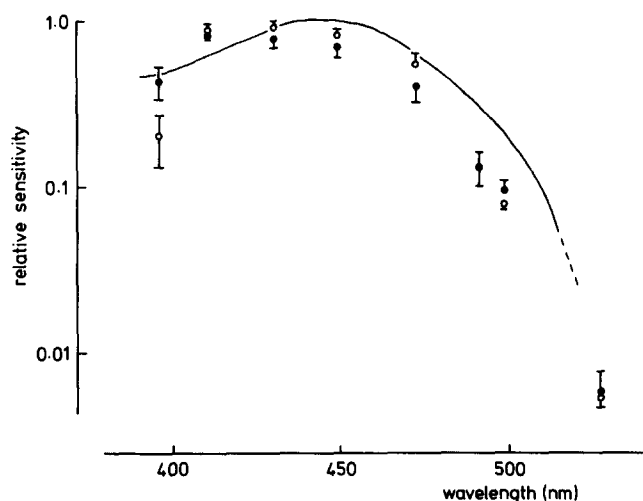

FIGURE 7.   Action spectrum of electrical responses (filled circles) and of ΔQ_{O_2} (open circles) after short (40 ms) monochromatic light flashes, plotted on a logarithmic scale. Error bars show standard errors ($n = 3$–7). The data for the action spectrum of ΔQ_{O_2} are the same as those of Fig. 3 after averaging and replotting. The curve is the normalized photosensitivity curve of the drone R (see Fig. 1).

hardly greater than noise levels. In some experiments, flashes up to 900 ms long were needed to product the effect.

Fig. 8 shows an example from an experiment where the effect was very pronounced. This preparation had been adapted with orange light to convert any M present to R, and was then stimulated by a series of unattenuated monochromatic light flashes 300 ms long. The figure shows the ΔQ_{O_2} after unattenuated 410-nm and 527-nm flashes. The former produced a ΔQ_{O_2} that returned to the baseline in 20 s (curve 1). The ΔQ_{O_2} after the 527-nm flash was smaller, even though the light intensity was eight times higher, because of the much lower absorbance of R at 527 nm, but it returned to the baseline in <10 s (curve 2). The difference in the kinetics of the two responses became clear after normalization (dotted curve): the time courses of the rising phases were seen to be very similar, whereas the response after the 410-nm flash had a very much longer falling phase.

The change in kinetics of the second, smaller response in Fig. 8 was not due to depletion of R by previous flashes, since, as mentioned in the Methods (and see the Appendix), these flashes are expected to convert only a few percent of R to M. Also, a similar shortening of ΔQ_{O_2} was found when the order of the flashes was reversed or when an intermediate adaptation with orange light was used to reconvert any M produced back to R.

The shortening of ΔQ_{O_2} was most significant for long light flashes of wavelengths 500 and 527 nm. Because at these wavelengths the drone M is most sensitive (Fig. 1), it might be that the effect is due to photoconversion of M to R. This idea was tested by applying double flashes as described in the next section.
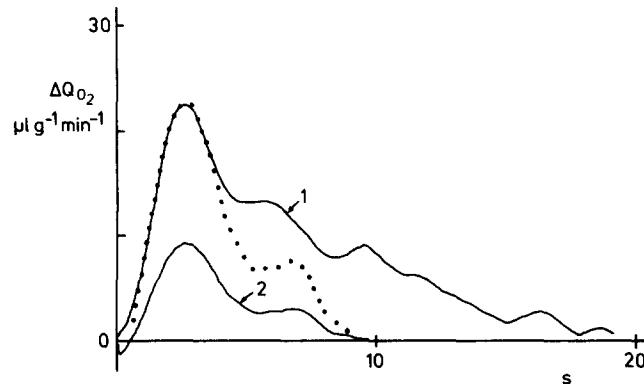

FIGURE 8. Change in kinetics of ΔQ_{O_2} after long monochromatic light flashes. Continuous curves are superimposed responses of ΔQ_{O_2} after 300-ms unattenuated flashes of wavelengths 410 (1) and 527 (2) nm. The relative intensity of the two flashes was 0.77 log units (see Methods; the 527-nm flash was more intense than the 410-nm flash). The points represent response 2 after normalization to the same peak amplitude as response 1. The apparently biphasic form of response 2 is an artifact arising from noise in the original recording.

ΔQ_{O_2} in Response to Double Light Flashes

Figs. 9 and 10 compare the ΔQ_{O_2} response after stimulation with unattenuated violet light with the ΔQ_{O_2} after double stimulation with unattenuated violet and green light. For these combinations, and after adaptation to orange, it was regularly found that the double flashes produced a smaller total ΔQ_{O_2}, and that the difference was in the falling phase of the response, which occurred faster. The rising phases of the responses to the single and double flashes were very similar.

The shortening of ΔQ_{O_2} after a double flash appears to be due to the photoconversion of M to R. The change in kinetics was only found when a green, 500- or 527-nm flash was added to a 410-nm flash. Both these double flashes produced a significant amount of reconversion to M, but the former shortened the response even though it produced a larger net change in the amount of R (see Table I). Thus, it is the reconversion of M to R and not the amount of M produced that gives rise to the effect shown in Figs. 9 and 10. With other wavelengths for the
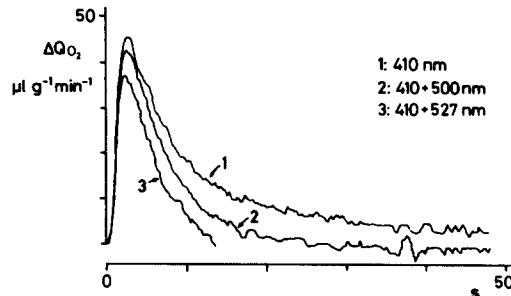
FIGURE 9. Inhibition of the falling phase of ΔQ_{O_2} during double light flashes. Superimposed responses of ΔQ_{O_2} after a single flash of 410 nm (1) and after double flashes of 410 and 500 nm (2) and 410 and 527 nm (3). See Table I for the estimated photoconversions by these flashes. Flash duration, 300 ms; delay between the onset of the two flashes, 50 ms. Relative flash intensities were −0.77, −0.13, and 0 log units at 410, 500, and 527 nm, respectively (see Methods).
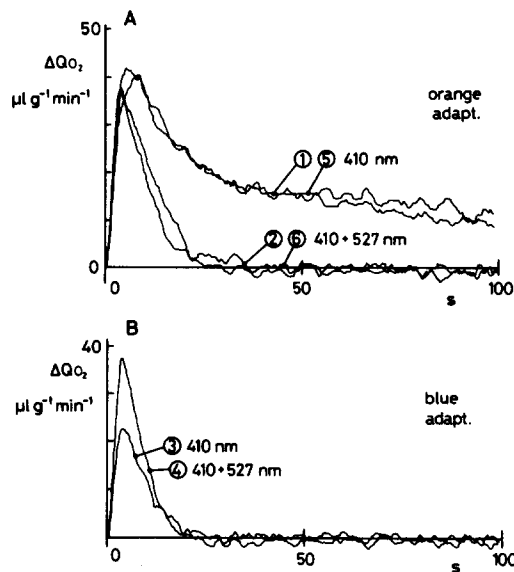

FIGURE 10. Inhibition of the falling phase of ΔQ_{O_2} depends on the initial state of the photopigment. (*A*) Responses 1 and 2 were recorded after adaptation to orange light (wavelengths >530 nm, producing >99% R). The ΔQ_{O_2} response to a violet flash at 410 nm was very long-lasting in this preparation. The ΔQ_{O_2} after a double flash of violet (410 nm) and green (527 nm) had a similar rising phase, but a much smaller falling phase. (*B*) After adaptation to violet light at 410 nm (producing 14% R and 86% M), responses 3 and 4 were recorded. The ΔQ_{O_2} after a violet flash was smaller and not long-lasting, and the response to a double flash was greater than the response to a single flash. This dependence of the time course of ΔQ_{O_2} on the initial state of the photopigment is perfectly reversible, as shown by responses 5 and 6 of *A*, which were obtained after readapting the slice with orange light. Flash duration, 600 ms; delay between the onset of flashes, 50 ms. Relative light intensity, 0.77 log units (the 527-nm flash was more intense than the 410-nm flash; see Methods).

second of the double flashes, there was either no effect or, for a shorter wavelength (430 nm), there was an increase in the response over that with the 410-nm filter alone (because of a greater R-to-M conversion, with very little M-to-R reconversion).

To further test the idea that the shortening of ΔQ_{O_2} is due to M-to-R photoconversion, the double-flash experiment was repeated after changing the initial state of the photopigment. After adaptation to violet light, a mixture of 14% R, 86% M was obtained. In this case, both the violet flash and the green flash were expected to produce R-to-M and M-to-R conversions. The double-flash experiment no longer showed an inhibition of ΔQ_{O_2}. In contrast, the response to a combined violet and green flash was now larger than the response to a single violet flash (Fig. 10B). Also, the responses after both the single and the double flashes had short time courses, in agreement with the idea that M-to-R conversion produces a shortening of the time course of ΔQ_{O_2} (Fig. 10).

After adaptation to white light, which is expected to produce a mixture of ~60% R and 40% M (see Appendix), the results for the double-flash experiment were variable. In two experiments, a double flash of violet and green light produced a reduced and shortened ΔQ_{O_2} when compared with the response after a single violet flash, which was similar to the result after orange adaptation (Figs. 9 and 10A). In a third experiment, there was an increased response to the double flash compared with the single flash, which was similar to the result after violet adaptation (Fig. 10B).

Fig. 10 also shows that these changes in the ΔQ_{O_2} responses due to manipulation of the initial state of the photopigment were completely reversible, which is further evidence that the initial event stimulating metabolism in the drone retina is photoconversion of the visual pigment.

Parallelism between Ion Movements and ΔQ_{O_2} Responses

The results described above indicate a close relationship between ΔQ_{O_2} and receptor potentials in the drone retinal slices after monochromatic light flashes, in that the action spectra for both responses are similar (Fig. 7). Also, at least over a certain range of intensities, both show a similar dependence on light intensity (Figs. 3 and 6). The same dependence was previously described for white-light flashes, although it was pointed out that only the receptor potentials saturate at high light intensities (Tsacopoulos and Poitry, 1982). In the previous section, we described changes in the kinetics of ΔQ_{O_2} that are dependent on the color of the light stimulus. It was therefore of interest to examine whether there are similar changes in the light-stimulated electrical responses and ion movements. For these experiments, intracellular [K$^+$] changes were followed using a double-barreled K$^+$-sensitive microelectrode. The electrical responses were recorded from the reference barrel of this electrode. In the experiment illustrated in Fig. 11, after orange adaptation, a violet flash of 30 or 300 ms duration produced a rapid decrease in intracellular [K$^+$] of 4–8 mM. For the shorter violet flash, recovery to the baseline occurred in ~30 s. For the longer flash, however, intracellular [K$^+$] remained at a low level for at least 30 s, and then returned only slowly to the baseline. Thus, strong violet flashes, producing mainly R-to-

A

Kᵢ⁺ 92 (mM) 84

Vm 0 (mV) -60

30s

B

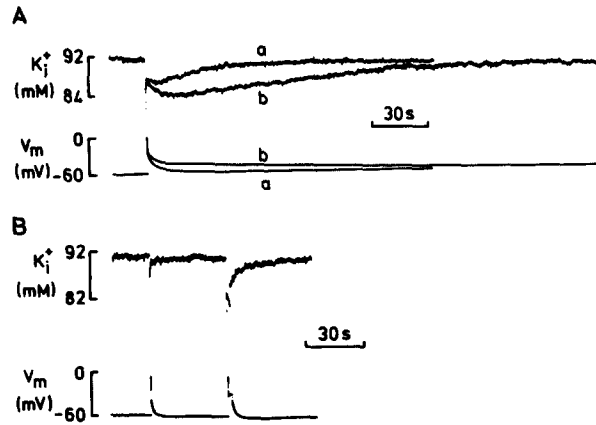Kᵢ⁺ 92 (mM) 82

Vm 0 (mV) -60

30s

FIGURE 11. Recordings showing long-lasting changes in ionic concentrations after strong violet flashes in an orange-adapted slice. The intracellular recordings from a photoreceptor cell were obtained using a double-barreled K⁺-sensitive microelectrode. The lower traces are membrane potential recorded from the reference barrel. The upper traces are intracellular K⁺ activity, with calibration from external standards. (A) In response to single flash of light, the membrane transiently depolarized and intracellular [K⁺] decreased from its dark level by ~6 mM before slowly recovering. Trace a was elicited by a 30-ms violet flash on an orange-adapted slice. Trace b was elicited by a 300-ms violet flash, also after orange adaptation. The longer violet flash produced a larger exit of K⁺ and a more prolonged afterpotential. (B) From the same cell, after adaptation to white light, a later recording shows responses to a 30-ms flash and a 10-s step of orange light (wavelengths >530 nm). After these stimuli, intracellular [K⁺] recovered rapidly to the baseline, even though the initial changes in K⁺ have about the same amplitude as in A.
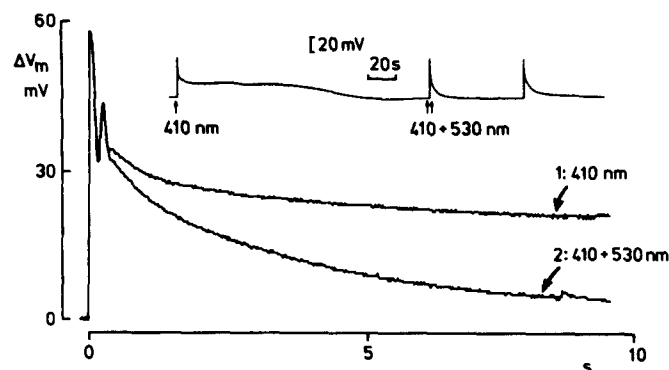
FIGURE 12. Electrical recordings from a photoreceptor cell in a slice of drone retina, showing shortening of the receptor potential after a double flash of violet and green light (2), in comparison with the response to a single violet flash (1). The inset shows a chart recording of the same responses on a slower time scale. Flash duration, 300 ms; delay between the onset of flashes, 50 ms.

M conversion in an orange-adapted preparation, can produce long-lasting changes in intracellular ionic concentrations. This is also true for the electrical responses, as seen in the long-lasting depolarizing afterpotential in the second pair of traces of Fig. 11A. The same cell was then adapted to white light and stimulated with orange flashes using the 530-nm cut-on filter. Although the sensitivity of R is low at 530 nm, the light intensity was sufficient to produce very similar changes in intracellular [K⁺] and similar electrical responses as after the violet flashes, which indicates that a similar R-to-M conversion occurred. However, because the sensitivity of M is high at 530 nm, significant M-to-R conversion is also expected. Fig. 11B shows that there are then no long-lasting aftereffects of the flash, either in intracellular [K⁺] or in the electrical responses. Similar results were found in five other cells.

Thus, the same monochromatic light stimulus that can produce a long-lasting ΔQ_{O_2} can also produce long-lasting changes in ionic concentrations and electrical responses. Furthermore, we note that the time courses of the changes in intracellular [K⁺] shown in Fig. 11A are slower than the typical ΔQ_{O_2} after the same stimulation (cf. Figs. 2B, 8, and 9; responses 1 and 5 of Fig. 10 were exceptionally long-lasting). This confirms and extends the previous finding (Tsacopoulos et al., 1983), using white-light stimuli, that the ΔQ_{O_2} after a light flash is faster than the ion pumping that is part of the recovery process after the flash.

Fig. 12 shows the result of an experiment where the amplitude of the afterpotential in response to a bright violet flash was reduced when a double flash of violet and green light was applied. In this cell, the afterpotential after a 300-ms unattenuated violet flash lasted for 100 s, whereas the depolarization after the double flash returned to the baseline after only 10 s. The phenomenon described above, that the ΔQ_{O_2} after a strong violet flash can be reduced by adding a green flash, is therefore also reflected in the electrical responses of the drone photoreceptor cells.

DISCUSSION

We have found that the action spectrum of the extra oxygen consumption (ΔQ_{O_2}) induced by a short monochromatic light flash presented to slices of the honeybee drone retina corresponds closely to the action spectrum of the receptor potential measured under the same conditions. This result indicates that the initial event for stimulation of ΔQ_{O_2} is very probably the same as the initial event for the induction of the receptor potential, i.e., photoconversion of rhodopsin (R) to metarhodopsin (M).

It is likely that the deviation of the observed action spectrum of ΔQ_{O_2}, and the action spectrum of the electrical responses, from the photosensitivity spectrum of the drone R is due to spectral effects of light scattering and absorption (and possibly reflections) in the retinal slices used in the experiments. We attempted to estimate these effects by measuring the transmission of slices of the drone retina at different wavelengths. The results indicated that 87–91% of the incident light is absorbed within a 300-μm-thick slice for wavelengths between 396 and 527 nm, and confirmed that the effective light source for R excitation within the slices is scattered light, since absorption by R molecules is estimated to be only ~1% for this thickness of retina (see Appendix). The absorption spectrum

of the slices, with a peak at ~420 nm, a fall-off toward the ultraviolet, and a less sharp fall-off at longer wavelengths, is very different from typical measurements of the extinction spectrum of pigment granules of insect retinas, which generally have a minimum at ~400 nm and a rising extinction at shorter and longer wavelengths (Langer, 1975). The application of a simple model to adjust our action spectra data to take account of the measured light absorption in the slices produces corrections that are consistent with the idea that these effects are the origin of the discrepancy with the R spectrum, but are not sufficient to account quantitatively for the discrepancies. The reason for this is probably that we are not able to include the contribution of screening pigment and other absorption in distorting the absorption spectra of the retinal slices.

The absorption spectrum of riboflavin and the action spectra of the photoreduction of cytochrome c and photostimulation of O_2 uptake in solutions of riboflavin and cytochrome c peak at 450 nm (Schmidt and Butler, 1976). These spectra are similar to the action spectrum for ΔQ_{O_2} in the drone retina, but we think it unlikely that this similarity is relevant to the physiology and metabolism of drone photoreceptors for the following reasons: (a) the action spectrum of light-induced ΔQ_{O_2} of *Calliphora erythrocephala* photoreceptors measured in the steady state by Hamdorf and Langer (1966) closely matched the absorption spectrum of the R of the retina; (b) in *Balanus eburneus* photoreceptors, a single flash of light at 530 nm induces a much higher ΔQ_{O_2} than a flash at 410 nm, which is expected if the amplitude of ΔQ_{O_2} is linked to the phototransformation of R to M in these photoreceptors (Poitry, S., and H. Widmer, personal communication). Thus, in three different invertebrate photoreceptors with different visual pigments, the action spectra of light-induced ΔQ_{O_2} have maxima at different wavelengths, and only in the drone is this close to the maximum of the riboflavin/cytochrome c system. Consequently, the possibility that stimulation of ΔQ_{O_2} is caused by light absorption by a pigment other than rhodopsin becomes unlikely.

This statement is further supported by the findings of our experiments in which the photoreceptors were stimulated by two monochromatic light flashes. When a long violet flash, transforming ~6% of R to M, was presented to the retina, the time course of the descending phase of ΔQ_{O_2} was much prolonged, and this change in the kinetics did not occur if a second flash of green light was also presented. In parallel experiments using the same stimulation, measurements of intracellular [K⁺] simultaneously with measurements of the receptor potentials showed that, after a strong violet flash, the recoveries of intracellular [K⁺] and of the slow repolarizing phase of the receptor potential were also much slower than after a brief violet flash, and could also be shortened by superimposing a green flash on the violet flash. This indicates once again that both the metabolism and function of the photoreceptors depend on the phototransformation of the same rhodopsin visual pigment.

There must be a series of chemical reactions triggered by phototransformation of R to M that underlie both ΔQ_{O_2} and transmembrane ion movements. The increase in the duration of ΔQ_{O_2} induced by a long violet flash means that, after such a flash, these chemical reactions can no longer occur in a straightforward way, presumably because of production of a saturating quantity of a molecule,

which is probably M. The hyperproduction can be reversed by phototransform-
ing M back to R, and not by a reduction in the amount of M produced by the
flash, which suggests that the formation by light of M molecules is a central step
in the chain of reactions that leads to the stimulation of O_2 consumption. The
presence of M molecules remaining after a flash of light has previously been
shown to determine the latency and amplitude of the electrical response of
invertebrate photoreceptor cells (Hamdorf and Kirschfeld, 1980) and the fre-
quency of primary events in these cells, the spontaneous quantum bumps (Lisman,
1985).

Recently, Lisman (1985) and Stern et al. (1985) reported that in *Limulus*
median and ventral photoreceptors, the rate of spontaneous quantum bumps
was higher when the M concentration was high, and that the high rate of
spontaneous quantum bumps recorded in a metabolically poisoned photoreceptor
could be reversed by intracellular injection of ATP. These results suggest that
ATP and therefore metabolism may be necessary to disactivate the M* molecules.
Similar, though more indirect, evidence for this hypothesis was provided earlier
by Wong et al. (1976) from work on living *Drosophila*. Lisman (1985) has
extended this hypothesis by suggesting the involvement of M* phosphorylation
by ATP in the phenomenon of long-lasting afterpotentials. He predicted that,
after a bright light stimulation, multiple phosphorylation of the highly concen-
trated M* could imply a formidable use of ATP. Earlier work in the drone
provided evidence that the triggering stimulus for the light-induced ΔQ_{O_2} is not
the increase in the working of the Na pump (Tsacopoulos et al., 1983). This
hypothesis is supported by other findings showing that 2 s after a single bright
flash of white light, there is an increase in the concentration of ATP in the drone
retina (Coles et al., 1984). Consequently, it appears unlikely that ADP produced
by the working of the Na pump, or by other ATP-consuming processes induced
by light, acts as the control for O_2 consumption.

It is not excluded, however, that a change in ATP turnover induced by light
affects the respiratory rate. Thus, an increase in the Na pumping rate could
explain the change in the kinetics of the descending phase of ΔQ_{O_2} that we find
to occur after a long violet flash, which transforms ~6% of R to M (see Appendix).
It seems unlikely, however, that R phosphorylation is involved in this change in
kinetics, because this phosphorylation involves only a small amount of ATP.
Assuming classic stoichiometry, that one O_2 molecule consumed produces six
ATP molecules, and taking the integral of ΔQ_{O_2} (mean value, 4.8 μl g^{-1} in four
experiments), we calculate that, after a strong violet flash such as that of Fig. 8,
there is production by the mitochondria of 2.6 mM ATP in the photoreceptors
(cf. Tsacopoulos et al., 1983); when the green flash was combined with the violet
flash and the ΔQ_{O_2} response was shortened, 1.6 mM ATP was produced. In four
experiments where the inhibitory effect of the green flash was very significant,
the reduction in ΔQ_{O_2} caused by M-to-R conversion ranged from 0.4 to 1.5 mM
ATP. This is about three orders of magnitude higher than the expected reduction
in Δ[ATP] if either two (Paulsen and Hoppe, 1978; Vandenburg and Montal,
1984) or even five (Lisman, 1985) ATP are used to phosphorylate one M, since
we estimate that no more than 2% of the photopigment is reconverted from M

to R during the stimulus, and that the photoreceptors contain ~8 μM R (see Appendix). Interestingly, the magnitude of the discrepancy is about the same as the proposed amplification factor for discrete events in invertebrate photoreceptors (Wong, 1978; Bacigalupo and Lisman, 1983), which implies that the factor controlling metabolism, at least in the drone photoreceptor, is present at a fairly early stage in the phototransduction process.

It is possible that ions, possibly Ca, could be involved in this amplification, since a single bright flash can produce a >10-fold increase in the intracellular Ca concentration (Levy and Fein, 1985). There is growing experimental evidence suggesting that Ca ions play a role in the regulation of the light-induced metabolism of invertebrate photoreceptors, but it is too early for more fruitful discussion at the present.

APPENDIX

Concentration of Photopigment in Drone Retinal Slices

From Fig. 7 of the paper by Muri and Jones (1983) and from the theory described in the Appendix of that article, the absorbance for light passing along the rhabdom owing to the photopigment when it is >99% R is calculated to be 0.16 log units (100 μm)$^{-1}$ at the peak wavelength of the drone R absorption (446 nm). For this calculation, the relative quantum efficiency for the invertebrate R-to-M photoconversion was taken to be 0.71 (Cronin and Goldsmith, 1982). An absorption of 0.16 log units (100 μm)$^{-1}$ is equivalent to an average pigment concentration of 0.4 mM, if the molar extinction coefficient is 40,000 liter mol^{-1} cm^{-1}, as measured for crayfish R in solution (Larrivee and Goldsmith, 1982). This calculation neglects the effect of dichroism of the drone rhabdoms, but the correction for dichroism is relatively small. (The dichroism of the glutaraldehyde-fixed rhabdoms used for microspectrophotometry has not been measured, but if the microvilli are aligned in equal amounts orthogonally, and the R chromophores are aligned so that each microvillus has a dichroic ratio of 9:1 [cf. Shaw, 1969], the R concentration is overestimated by a factor of only 1.36.) An R concentration of 0.4 mM is only about half of that estimated in a similar way for crayfish rhabdoms (Goldsmith and Wehner, 1977), but the uncertainties involved are high, and there are also wide individual and seasonal variations in the density of photopigment in drone rhabdoms (Muri, R. B., unpublished data).

Inspection of light and electron micrographs of the drone retina (Perrelet, 1970; Muri and Jones, 1983) indicates that the rhabdoms occupy ~1% of the total volume, so the absorbance owing to the photopigment, in the R state, in 300-μm slices illuminated transversely is ~0.005 log units (again neglecting effects of dichroism). Thus, R is expected to absorb ~1% of the incident light (at its absorption peak). The mean concentration of R in the photoreceptors is ~8 μM, since these occupy about one half of the total volume (Coles and Tsacopoulos, 1979).

Light Adaptation

The equilibrium fraction of photopigment in states R or M during adaptation with monochromatic light is given by (Stavenga, 1975; Hochstein et al., 1978):

$$f_R = \frac{K_M}{K_R + K_M} \text{ and } f_M = \frac{K_R}{K_R + K_M},$$ \hfill (1)

where K_R and K_M are the photosensitivities of states R and M. At 410 nm, the values of

K_R and K_M for the drone are 6.3 and 1.0×10^{-17} cm^2 photon^{-1} (Fig. 1). From Eq. 1, adaptation with violet light at 410 nm therefore produces a steady state with an R fraction of 0.86 and an M fraction of 0.14.

If the adapting light is not monochromatic, the equilibrium fraction of pigment in state R is given by

$$f_R = \frac{\int K_M(\lambda) \cdot I(\lambda) \cdot d\lambda}{\int K_R(\lambda) \cdot I(\lambda) \cdot d\lambda + \int K_M(\lambda) \cdot I(\lambda) \cdot d\lambda}, \tag{2}$$

with a similar equation for f_M obtained by replacing R by M and M by R. At wavelengths >530 nm, the photosensitivity of R is always <1% of that of M, so adaptation with a 530-nm cut-on filter produces a steady state with >99% R. For adaptation with white light, the steady state mixture can be estimated on the assumption that the white-light spectrum is flat between 350 and 650 nm. Integration of the photosensitivity spectra of Fig. 1 then gives an equilibrium with an R fraction of 0.6.

The time course of the approach to a steady state during monochromatic light adaptation of a simple bistable photopigment is known to be exponential with a time constant equal to the inverse of the product of the light intensity and the sum of the photosensitivities of the two states at the adapting wavelength (Hochstein et al., 1978; cf. Eq. 3 below). For violet light at 410 nm, with photosensitivities of R and M as given above and taking the effective light intensity as equal to the incident light intensity, 1.5×10^{15} photons cm^{-2} s^{-1}, the calculated time constant is 9.1 s. The orange cut-on filter used for adaptation from M to R was measured to have 1% transmission at 530 nm, 50% at 540 nm, and a fairly flat transmission from 550 up to 750 nm, which was the infrared cut-off for the experimental setup. The incident light intensity with this filter was 2.4×10^{16} photons cm^{-2} s^{-1}, i.e., ~1.2×10^{14} photons cm^{-2} s^{-1} nm^{-1}. Combining this intensity with the transmission of the filter and the photosensitivity of M above 530 nm (Fig. 1) and integrating over wavelength gives a time constant of 2.8 s during adaptation with orange light.

Light Stimulation

For a simple bistable photopigment, the fraction of the pigment in state R at time t after the onset of light of intensity I is given by (Stavenga, 1975; Hochstein at al., 1978; see Hillman et al., 1983):

$$f_R(I \cdot t) = f_R(\infty) + [f_R(0) - f_R(\infty)] \cdot \exp[-(K_R + K_M) \cdot I \cdot t], \tag{3}$$

where $f_R(\infty)$, equal to $K_M/(K_R + K_M)$, is the equilibrium fraction of R after adaptation at the same wavelength as the flash, and $f_R(0)$ is the starting fraction of the pigment in state R. The fraction of the pigment in state M is given by the similar equation obtained by replacing R by M and M by R. Eq. 3 allows calculation of the net fractional transfer of photopigment molecules from R to M (or M to R) during a flash, but it does not allow calculation of the fraction that is transferred in any one direction. Also it does not allow calculation of the fraction of molecules transferred if the stimulus spectral composition is the same as that of the previous adapting light, since the net transfer is then zero.

Defining f_{M*} as the fraction of photopigment molecules transformed from state R to state M during a flash, the fractional rate at which molecules are being transformed at any time t is

$$\frac{d}{dt}(f_{M*}) = K_R \cdot I \cdot f_R(I \cdot t). \tag{4}$$

The total fraction of molecules converted from R to M by a flash of duration d is given by combining Eqs. 3 and 4 and integrating over time. After rearrangement,

$$f_{M*} = K_R \cdot f_R(\infty) \cdot I \cdot d + [1 - f_R(\infty)] \cdot [f_R(0) - f_R(\infty)] \cdot \{1 - \exp[-(K_R + K_M) \cdot I \cdot d]\}. \quad (5)$$

A similar equation, with R replaced by M, gives the fraction of photopigment molecules converted from M to R during the flash. These equations hold for monochromatic light stimuli. Extension to stimuli that are not monochromatic is straightforward: as in the previous section, the photosensitivities must be replaced by integrals over the wavelength of the product of photosensitivity and light intensity.

For white-light flashes on a white-adapted preparation, the calculation of f_{M*} involves only the first term of Eq. 5, but integration over wavelength must be included:

$$f_{M*} = f_R(\infty) \cdot \int K_R(\lambda) \cdot I(\lambda) \cdot d\lambda \cdot d. \quad (6)$$

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