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Visual response in barnacle photoreceptors is not initiated by transitions to and from metarhodopsin

ABSORPTION of a photon by the visual pigment of a photoreceptor results in a cascade of changes in the pigment molecule. At some time during the later stages of this cascade, a visual response appears, in the form of a change of the ionic conductance of the cell. In order to understand the mechanism of the process coupling the pigment changes to the modulation of the conductance, one should know from which state(s) or transition(s) of the pigment cascade the coupling process originates. In the vertebrate, it is commonly assumed[1,2] that the fourth transition in the cascade, that from the state called metarhodopsin I to the state metarhodopsin II, is the predominant source of the coupling, mainly because this is the first transition involving a major molecular conformational change and the last temporally before the appearance of the visual response. Here we show that in the barnacle photoreceptor, transitions to and from the state called metarhodopsin (although its relation to vertebrate metarhodopsin is unclear) cannot be primarily involved in originating the coupling process, which must arise from earlier stages, not later than the second transition of the cascade.

In many invertebrate visual pigments, the metarhodopsin state is long lived and so can easily be photo-excited. We have shown that the barnacle pigment transition scheme contains return loops, so that most of the thermal transitions are activated in some degree by photoexcitation of both rhodopsin (Rh) and metarhodopsin (M) (see Fig. 1). By demonstrating that the shape of the late receptor potential (LRP) action spectrum is close to that of the photosensitivity spectrum of rhodopsin and is unaffected by shifts in the rhodopsin–metarhodopsin population ratio, we are able to exclude as major sources of the coupling to the LRP response all metarhodopsin-activated transitions and all states fed by these transitions. The only remaining candidates for the coupling are the phototransition from rhodopsin and its immediate thermal successor—the first rhodopsin photoproduct state.

Our previous analysis of the transition scheme of the barnacle photo-receptor visual pigment was based on early receptor potential (ERP) observations[3]. This scheme is shown in Fig. 1, omitting photo-transitions from the thermally unstable states since the intensities used in this experiment were always so low as to make them negligible. Figure 1 shows that photo-excitation of metarhodopsin strongly activates all of the transitions except Rh⇝B and B→C. If any of the metarhodopsin-activated transitions were substantially coupled to the LRP response, the action spectrum of the LRP should either be the metarhodopsin photosensitivity spectrum (for coupling from transitions M⇝E and E→F) or a weighted sum of the spectra of metarhodopsin and rhodopsin (transitions C→M, C→F, F→C and F→Rh). For each transition putatively coupled to the LRP, the LRP action spectrum may be predicted from the transition scheme and parameters shown in Fig. 1 and the initial population ratio of the rhodopsin and metarhodopsin states. This ratio depends on the wavelength of the light to which the preparation has been adapted and may either be calculated for this wavelength from the transition scheme

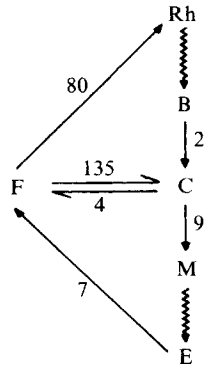

Fig. 1 The transition scheme of the barnacle visual pigment. The values are the thermal time constants of the transitions in ms at 20 °C. Rh, rhodopsin; M, metarhodopsin. Smooth arrows indicate thermal transitions, zigzag arrows phototransitions. The scheme and constants are those derived from early receptor potential observations[3] except that phototransitions from unstable states are here omitted since their effects are small at the adapting light intensities used in the present experiment, and except for a small temperature correction (23 °C→20 °C) of the thermal constants.

and parameters or may be directly measured using the ERP[4]. In this experiment we have measured the LRP action spectra following saturating adaption at two wavelengths which give the largest population difference; we have used ERP data to determine the rhodopsin-metarhodopsin population ratio in each case; and with those ratios we have calculated, for each putatively coupled transition, the expected LRP action spectrum. By comparing the positions of the observed LRP action spectra and the difference between them with these predictions, we are able to exclude all of the metarhodopsin-related transitions and states as dominant couplers. (LRP action spectra have previously been measured in the barnacle with various adaptations, but in conditions which do not permit determination of the pigment state during the measurements[5].)

All measurements were intracellular recordings from excised lateral ocelli of *Balanus amphitrite* at 20 °C. Cells were adapted and stimulated by light from a quartz-iodide lamp passed through Balzers broad band (K2 or K3 for 'blue' and K6 for 'red') and narrow band (B 40) interference filters respectively.

In the barnacle, the rhodopsin and metarhodopsin spectra have peaks at about 532 and 495 nm respectively[6–8]. Red adaptation maximises the metarhodopsin population and blue the rhodopsin. According to ERP measurements, the rhodopsin:metarhodopsin ratios were about 80:20 and 5:95 following blue and red adaptation respectively.

The LRP action spectra following red and blue adaptation were measured by determining, at each wavelength, the intensity of a brief (30 or 300 ms) flash of light required to elicit a criterion small (3 mV or less) LRP response. This was done only when the sensitivity of the cell had reached (or nearly reached) a plateau, which sometimes took 2 or 3 h after the bright adapting illumination. The resulting action spectra, the wavelength dependences of the reciprocal of the amount of light needed to produce a criterion response at each wavelength, are displayed in Fig. 2. Both spectra are normalised by placing at 1 the peaks of smooth curves fitted to the points. (Observations on the ratio of the absolute sensitivities and the relation of this ratio to the rhodopsin population have been briefly reported[9,10] and will be presented in detail elsewhere.)

Also shown in Fig. 2 for comparison are smooth curves matched to the ERP spectra[6,7] (photometrically confirmed[8]) of rhodopsin (curve *a*) and metarhodopsin (curve *b*), together with a curve which is a 50:50 weighted sum of the two 'pure' curves. All three curves are also normalised to 1 at their peaks.

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It is clear that both of the LRP spectra are, to a high confidence level, much closer to the rhodopsin ERP spectrum than is the intermediate curve; in other words, the metarhodopsin contribution to these spectra is appreciably less than 0.5. Furthermore, we estimate that the difference between the metarhodopsin contributions to the two LRP spectra is less than about 0.2 (much smaller scatter and possibility of systematic error; the bulk of the uncertainty in the ERP–LRP comparison arises from the ERP measurements).

For comparison with these experimental limits, we present in Table 1 the predicted contribution of the metarhodopsin spectrum to the LRP spectrum on the assumption that each of the transitions listed is the sole source of coupling to the LRP. It is clear that the only transitions not excluded by the experimental results are Rh⇝B and B→C. Furthermore, since the transition rate from a particular state is proportional to the population of that state, all states but B are also excluded. The coupling to the LRP response must therefore arise predominantly from one or both of these transitions or from state B. Upper limits to the possible contributions from other states and transitions can be calculated from Table 1.

Having shown that metarhodopsin does not contribute to the LRP, we stress that it does have a physiological effect: excitation of metarhodopsin depresses or prevents the induction of prolonged depolarising afterpotentials[11]. It may also influence the LRP at stimulus intensities higher than those used here[11].

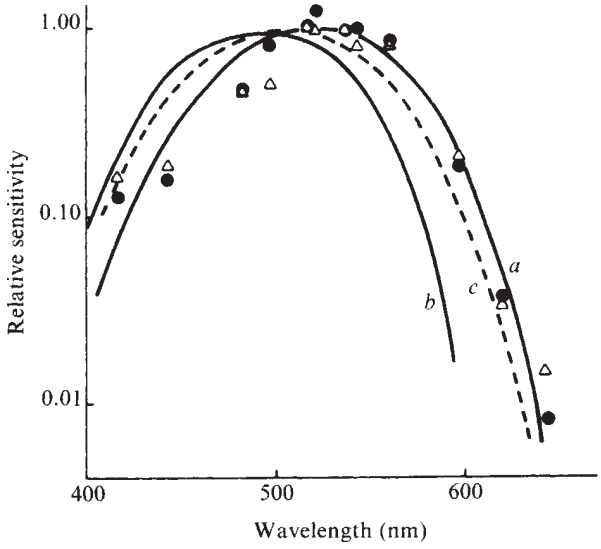
Note that both of the transitions allowed as couplers are much faster (<3 ms) than the LRP latency. The coupling process must therefore involve a slow intermediate (non-pigment) process.

Table 1 Calculated contributions of the metarhodopsin spectrum to the LRP spectrum for various transitions possibly coupled to the LRP

Transition	Fraction of photoexcitations going through transition from Rh	M	Metarhodopsin contribution to LRP spectrum following adaptation by Blue	Red	Δ
Rh⇝B; B→C	1	0	0	0	0
C→M; C→F	0.4	0.15	0.92	0.13	0.79
F→C; F→A	0.6	0.85	0.98	0.36	0.62
M⇝E; E→F	0	1	1	1	0
Experimental	—	—	<0.5	<0.5	<0.2

The first two numerical columns give the fractions of rhodopsin and metarhodopsin photoexcitations (respectively) which pass through the transitions indicated, as calculated from Fig. 1. The metarhodopsin contributions to the LRP spectrum after blue and red adaptation are presented in the next two columns. These are calculated from the first two columns as follows: the metarhodopsin fraction divided by the sum of the two fractions is multiplied by the relative metarhodopsin populations after blue and red adaptation (0.2 and 0.95) and by the ratio[8] of the peak photosensitivities of metarhodopsin and rhodopsin (1.6). The final column gives the differences between the values of the two preceding columns and indicates the expected spectrum shift resulting from adaptation. The final row gives the experimental limits.

Fig. 2 Experimental and theoretical late receptor potential (LRP) action spectra. The points are the LRP sensitivities (reciprocals of numbers of photons needed to elicit criterion LRP responses) following saturating red (●) and blue (△) adaptation of the cells. Each point is the average of measurement from two cells. The curves are smooth fits (by eye) to the early receptor potential action spectra of rhodopsin (*a*) and metarhodopsin[6,7] (*b*) (with small wavelength corrections from recalculations) as confirmed by photometry[8]. Curve (*c*) is a 50:50 sum of (*a*) and (*b*). All three curves as well as the experimental points are normalised to 1 at their peaks. Since red adaptation shifts most of the pigment into the metarhodopsin state, while even blue adaptation leaves some in that state, any substantial coupling of metarhodopsin-related transitions or states to the generation of the LRP should exhibit itself in a leftward shift (from the rhodopsin curve) of the triangles and, much more strongly, the circles. The smallness of any such shifts is interpreted in the text as showing that, of the states and transitions of the scheme of Fig. 1, only the transitions Rh⇝B and B→C and the state B can be substantially coupled to the generation of the LRP.



An alternative approach to the location of the transduction coupling would be an examination of the dependence on rhodopsin population of the LRP sensitivity at a particular wavelength or band of wavelengths. If the dependence is linear at wavelengths at which metarhodopsin has an appreciable sensitivity, any contribution from metarhodopsin-excited transitions is excluded (except for unlikely accidental cancellations). Such a linear dependence was indeed found in the octopus, fly and moth[12]. The detailed pigment schemes are, however, not known in these animals. Nonlinear dependences have been observed in the barnacle[9,10] and in the blowfly[13] but the spectral measurements described above exclude a metarhodopsin contribution as the source of the nonlinearity in the barnacle and the absence of such measurements allows no conclusion for the blowfly.

The various pigment states in the barnacle have not been studied biochemically; our results provide considerable incentive for such a study. If the transition to metarhodopsin is shown to be the first in the chain to involve a major conformational change, as in the vertebrate, that change will have been shown not to play a vital part in the transduction process in this system.

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Note added in proof: Strong and Lisman[14] have shown that metarhodopsin also does not contribute to light adaptation of this photoreceptor.

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Possible role of cyclic nucleotides in regulation of noradrenaline release from rat pineal through presynaptic adrenoceptors

PRESYNAPTIC α-adrenoceptors on noradrenergic nerve endings have been shown to mediate a negative feedback mechanism which leads to inhibition of transmitter release during depolarisation induced by nerve stimulation or potassium[1–3]. On the other hand, it is known that in some tissues activation of presynaptic β-adrenoceptors both *in vitro* and *in vivo* leads to an increase in transmitter release[4–8]. The α-adrenoceptors seem to operate by restricting the calcium available for the excitation–secretion coupling, and the facilitating effect of β-agonists on transmitter release seems to be mediated through an increase in the levels of cyclic AMP in noradrenergic nerve endings[7,8]. Recent reports[9,10] have provided electrophysiological evidence for a prejunctional role of cyclic nucleotides in neurotransmission. In the rat pineal gland a calcium-dependent presynaptic mechanism for the generation of cyclic GMP has been reported which might be linked to an α-adrenergic-like receptor[11]. In addition, there is evidence in this gland for an α-adrenergic presynaptic mechanism regulating noradrenaline release elicited by potassium[12]. Consequently, we decided to examine and report here our finding of a role of cyclic nucleotides in noradrenaline release from the rat pineal gland and the possible involvement of these substances in the regulatory mechanisms mediated through the α and β presynaptic adrenoreceptors.

For transmitter release studies we used a previously described technique[13]. Male rats (160–200 g) were killed by decapitation and their pineal gland removed immediately. For each experiment four pineals were placed in an open lucite cylinder with a piece of nylon mesh fitted to the bottom. The system was placed in a beaker containing 5 ml of Krebs bicarbonate solution at 37°C and was bubbled with a 95% O_2:5% CO_2 mixture. The endogenous noradrenaline stores were labelled *in vitro* by incubating the pineals with 0.5 μM (±)-7-^{3}H-noradrenaline for 30 min. When the spontaneous efflux of radioactivity had levelled off 50 min later, ^{3}H-neurotransmitter release was elicited either by a one-minute exposure to media containing 60 mM KCl or by a five-minute exposure to 3 μM tyramine (Fig. 1). During depolarisation with 60 mM KCl an equimolar reduction of NaCl was carried out to maintain the osmolarity of the medium.

In the controls, the fraction of the total tissue radioactivity released by the first exposure to potassium was 17.13 ± 1.05 ($\times 10^{-3}$) ($n = 74$) and the ratio obtained between the two consecutive stimulation periods (S_2/S_1) was 1.15 ± 0.10 ($n = 10$) (Table 1). In these experimental conditions, ^{3}H-noradrenaline release by potassium was found to be entirely calcium dependent[12]. Denervated pineal glands (seven days after bilateral superior cervical ganglionectomy) failed to retain ^{3}H-noradrenaline and did not release tritium when exposed to 60 mM potassium.

Exposure to oxymetazoline (10 μM), an α agonist that has been reported to be more selective for presynaptic α-receptors[3], produced a significant inhibition of ^{3}H-noradrenaline release. On the other hand, yohimbine (10 μM), a selective presynaptic α-blocking agent[3], increased transmitter overflow more than twofold (Table 1). When ^{3}H-noradrenaline release was elicited by exposure to 3 μM tyramine, the fraction of total tissue radioactivity released by S_1 was 38.15 ± 1.48 ($\times 10^{-3}$) ($n = 42$) and the ratio between two consecutive stimulation periods was 1.23 ± 0.10, $n = 8$ (Table 2). The effects of oxymetazoline and yohimbine on presynaptic α-receptors were selective for the release induced by potassium, as these drugs did not modify ^{3}H-neurotransmitter release elicited by exposure to tyramine (compare Tables 1 and 2).

To investigate the presence of presynaptic β-receptors in the noradrenergic nerve endings of the rat pineal gland, we tested the agonists isoprenaline (14 nM) and terbutaline (80 nM). Both drugs significantly enhanced ^{3}H-noradrenaline release

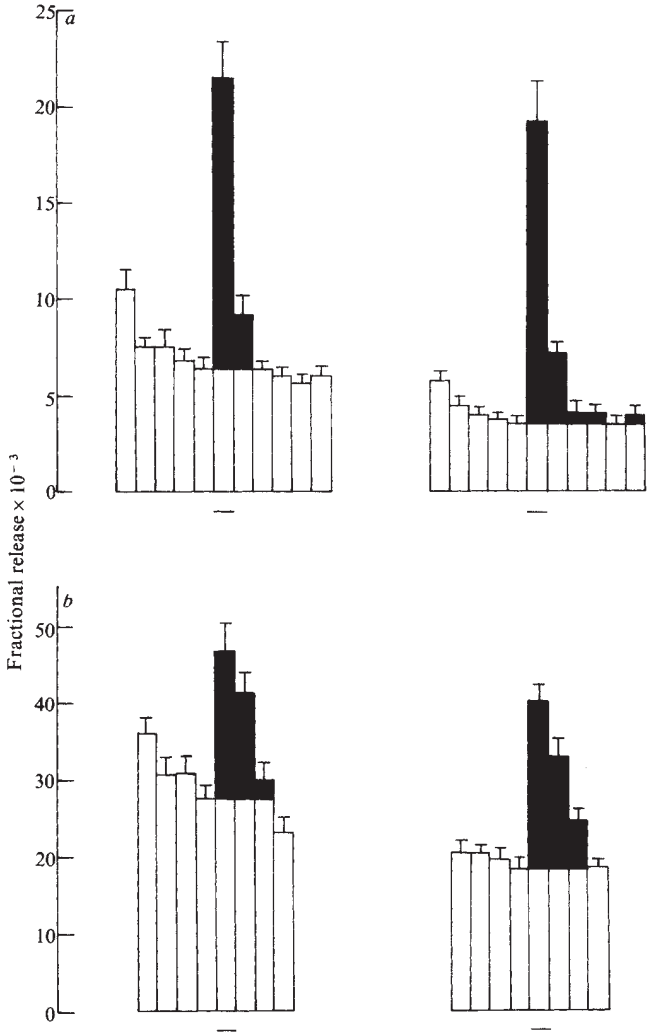

Fig. 1 Release of radioactivity from the rat pineal gland prelabelled with ^{3}H-noradrenaline during exposure to potassium or tyramine. Ordinates: fractional release ($\times 10^{-3}$): fraction of the total tissue radioactivity released per sample. *a*, Release of ^{3}H-noradrenaline evoked by 60 mM K^+ ($n = 10$). The interval between two consecutive potassium stimulations was 35 min. One-minute samples were collected. Tissue content at the end of the experiment was 252.2 ± 14.8 nCi per four pineals ($n = 10$). *b*, Release of ^{3}H-noradrenaline evoked by tyramine 3 μM ($n = 8$). The interval between both exposures to tyramine was 40 min. Five-minute samples were collected. Tissue content at the end of the experiment was 239.1 ± 25.8 nCi per four pineals ($n = 8$). Unshaded columns, spontaneous outflow of total radioactivity collected in each sample (1 min in *a* and 5 min in *b*). Shaded columns, increase in radioactivity induced by exposure to potassium or tyramine. The horizontal bars under the abscissae indicate the period of exposure to potassium (*a*) or tyramine (*b*). Mean values ± s.e.m. are shown; *n*, number of experiments per group.

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