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Absorption of Light by Metarhodopsin Modifies the Effect of a Conditioning Light on the Barnacle Photoreceptor*

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Abstract. We show that the effect of an adapting light on the sensitivity of barnacle photoreceptors depends on the direction of net pigment transfer [rhodopsin (R) to metarhodopsin (M) or reverse] occasioned by the adapting light. For stimuli giving no net pigment transfer the *state* of the pigment appears irrelevant, R → R having the same effect as M → M. With respect to these, R → M gives enhanced facilitation and M → R depressed facilitation. This suggests a correlation with the prolonged depolarising after-potential (PDA) and the anti-PDA, which follow R → M and M → R stimuli respectively. These effects appear mainly in less sensitive cells and for higher amounts of conditioning light – but still well within the physiological range and well below the threshold for PDA and anti-PDA induction. The special interest of these results is that they appear to be interpretable only by assuming that absorption of light by metarhodopsin exerts an effect on the stimulus coincident response (LRP), the first demonstration of such an effect.

Key words: Visual pigment – Photoreceptor – Metarhodopsin – Adaptation.

Introduction

In the invertebrate photoreceptor, the metarhodopsin state of the visual pigment is long-lived, so that normally the populations of the metarhodopsin (M) and rhodopsin (R) states of the pigment are both substantial during physiological stimulation. Does the presence or photoactivation of M contribute to the effect of a stimulus on the ensuing sensitivity of the cell? If so, does this effect arise only from a modification of the response to the conditioning stimulus, or is a separate process involved?

Four recent papers have related to the role of M in lobster (Barnes and Goldsmith, 1977), barnacle (Atzmon et al., 1978; Strong and Lisman, 1978) and *Limulus*

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(Lisman and Strong, 1978). These authors showed that under the conditions described in their papers, neither the presence of M nor the absorption of light by M affects the sensitivity of a cell. Light absorption: The action spectrum for light adaptation is the same as that for excitation and as the absorption and ERP spectra of R. Presence: The same response gives the same adaptation, independent of M population.

We present evidence that in the barnacle the presence of M is indeed irrelevant to the sensitivity-change effect of a conditioning light, but that under appropriate conditions the absorption of light by M has a strongly depressing effect on the cell sensitivity, or antagonizes the enhancing effect of absorption of light by R, even for nearly identical responses to the conditioning light. We conclude the article by attempting to reconcile the apparently conflicting observations.

Methods

The techniques were those of Hanani and Hillman (1976) except that coloured adapting and conditioning lights were used: Balzers K6 (red) and 443 nm (blue) interference filters. The measurements are illustrated in Figure 1: A series of very weak white test flashes (coloured flashes were also tried with no effect on the result) is presented to the cell. One is replaced by a bright conditioning flash. The ratio ($\equiv$ r) is measured of the average amplitudes of the 2nd, 3rd, and 4th post-conditioning test responses to the average pre-conditioning test response amplitude. The cell is initially adapted to saturating blue or red light. This light moves the bulk of the pigment to either the R or the M state. After at least 10 min of darkness, the test series is begun and a blue or red conditioning stimulus of a particular intensity and (brief) duration presented. If a blue conditioning light is presented to a blue-adapted cell no net pigment transfer takes place (the stimulus is "neutral"), the bulk of the pigment is, and remains, in the R state, and the experiment is labeled R $\rightarrow$ R. The red-after-red experiment is similarly labeled M $\rightarrow$ M. For red-after-blue and blue-after-red, net pigment transfers do occur, and these experiments are labeled respectively R $\rightarrow$ M and M $\rightarrow$ R. Note that R $\rightarrow$ M implies that the bulk of the pigment is initially in the R state, and that some of it is changed to M, but *not* that the final state is mainly M, since in most of the range of conditioning intensity investigated very little of the pigment is actually transferred. The actual amount of pigment transfer is known for each conditioning light from ERP measurements (Hillman et al., 1976).

Results

We have studied the dependence of the sensitivity-change parameter r on amount (intensity times duration) of adapting light and colour of adapting and conditioning light. Figure 2 shows the dependence of r on the logarithm of the relative amount of adapting light for the four combinations of adapting and conditioning wavelengths. The curves displayed are the averages of results from 16 cells. The R $\rightarrow$ M (red-after-blue) and M $\rightarrow$ M (red-after-red) curves have the same abscissa, since they refer to stimuli of the same wavelength; the same goes for M $\rightarrow$ R and R $\rightarrow$ R. The two

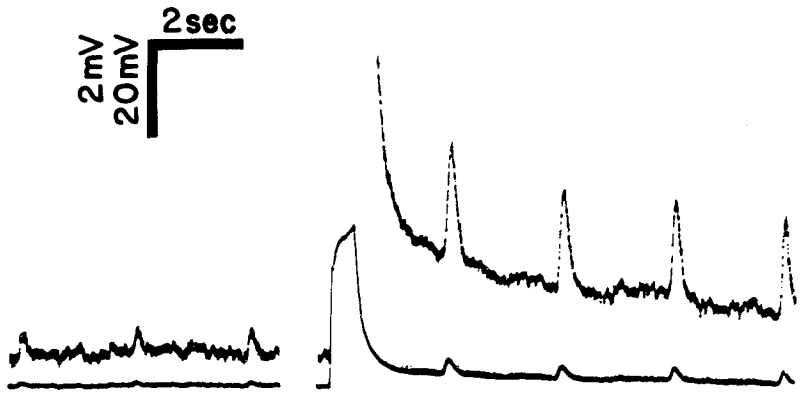

Fig. 1. The type of recording used to determine the effects of conditioning lights on cell sensitivity. Excised barnacle photoreceptors are bathed in seawater at 22° C. 10 min after saturating adaptation to red light (in this case), a series of 60 ms weak white test flashes is presented at 3 s intervals. One of the flashes is replaced by a bright red 600 ms conditioning light, so that the experiment is "M → M" (red-after-red). The ratio r of the amplitudes of the test responses after and before the conditioning flash is measured. (Upper trace gain ten times that of lower trace)

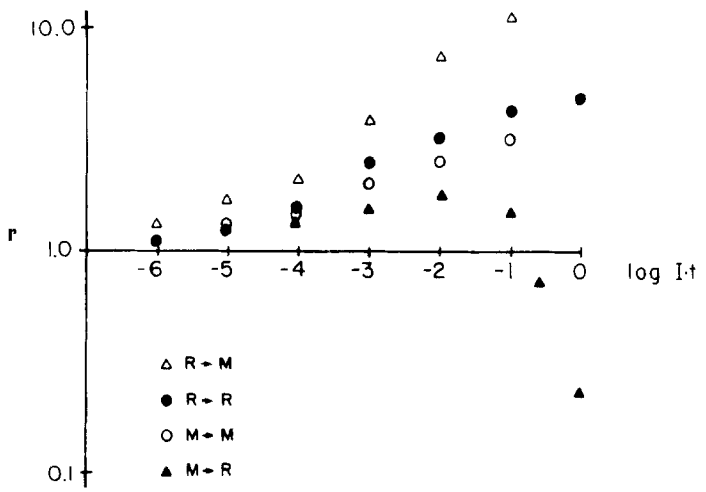

Fig. 2. The effect of a conditioning light on cell sensitivity as a function of the logarithm of the amount (intensity times duration) of the conditioning light. Each curve represents a combination of red or blue saturating prior adaptation and red or blue conditioning stimulation: Blue-after-blue is labeled R → R for the predominant pigment state, and so on. Each point is the average of measurements from 16 cells. The relation between the abscissa scales for the red and blue conditioning lights is fixed as described in the text. Log I = −3 corresponds to photoactivation of less than 0.1% of the pigment (by ERP calibaration). The divergence of the curves above log I = −3 is taken to indicate a contribution of photoactivation of metarhodopsin to the effect of the conditioning light in this light amount range. However, cells of very high sensitivity tended to show less – or no – divergence (all curves approaching the shape of the M → R curve) and were excluded from the averages shown here

abscissa scales are normalized to each other by equalizing the pigment absorption (R and M) for blue light in the blue-adapted state and red light in the red-adapted state: Blue and red neutral lights of the same nominal $\log I \cdot t$ will then do exactly the same thing to the pigment. (Improved pigment measurements suggest that all the open points may have been shifted about a half log-unit too far to the left.)

The observations showed a rather large scatter from cell to cell, a good part of which we were able to correlate with the dark-adapted sensitivity of the cell: In cells of very high sensitivity, there was a tendency for the R → M, R → R, and M → M curves to approach the M → R curve – that is, to show predominantly $r < 1$ effects. A few such cells, with sensitivity above an arbitrary criterion, were omitted from the averages of Figure 2.

Discussion

Hanani and Hillman (1976) have previously shown that the effect of a conditioning light on cell sensitivity depends on the amount of the conditioning light, on the external Ca^{2+} concentration, and on the initial (dark-adapted) cell sensitivity. Figure 2 of this article shows that quite different conditioning effects are seen also according to the ratio of light absorption by M and R – in other words, that absorption of light by M has a major effect. In particular, the observation that r is higher for R → R and M → M than for M → R, and is even higher for R → M, suggests either that absorption of light by M has a depressive effect on the cell sensitivity, or that absorption of light by R has an enhancement effect which is antagonised by absorption of light by M.

On the other hand, the similarity of the curves for the R → R and M → M experiments, which differ only in the "spectator" M/R population ratio, suggests that *presence* of M does not influence the cell sensitivity change. ("Spectator" refers to those molecules not absorbing light.)

Some (but not all) of the different effects of different pigment transfers on the cell sensitivity could in principle be due to different amplitudes of responses to the different pigment transfers. Preliminary observations by Laiwand et al. (private communication) suggest that such response differences do exist (in itself an interesting observation probably related to the present results) but that they are apparently too small to be significant in the present context.

Reconciliation of the present results with those of Strong and Lisman (1978), Lisman and Strong (1978), and Barnes and Goldsmith (1977) probably lies in one or more of three possible directions: In the latter two cases, a species difference; and in all three cases, possibly a difference in the intensity range and possibly in the selection of cells by dark-adapted sensitivity.

It should be pointed out, however, that the curves in Figure 2 diverge at a stimulus amount corresponding to light absorption by less than a tenth of a percent of the pigment, which is far below the threshold for the appearance of the PDA (prolonged depolarising after-potential) from R → M or the anti-PDA from M → R. The present results constitute the first clear demonstration of a role for M photoactivation in the stimulus-coincident response as opposed to the after-processes, but

may be taken to suggest that the contribution of M becomes substantial only at higher stimulus intensities, or pigment transfers. A cooperative process might be suggested.

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