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On the Implications of Bistability of Visual Pigment Systems*

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Abstract. The characteristics of different responses of invertebrate photoreceptors are reviewed. Invertebrate photopigment bistability has made possible the functional operational dissection of the pigment transition scheme. Outlasting the usual stimulus-coincident late receptor potential (LRP), additional antagonistic responses have been found: the prolonged depolarizing after-potential (PDA) arising from a net rhodopsin to metarhodopsin pigment shift, and a PDA-depression and an anti-PDA effect which arise from a reverse shift and cancel the PDA when induced during or closely before it. The characteristics of these after-effects and of the LRP are reviewed, analyzed and compared. Both potentials require rhodopsin activation and they share the characteristics of a common ionic conductance-change mechanism. However, for the LRP response to weak stimuli, no antagonistic metarhodopsin-dependent effect has been found analogous to PDA-depression and the anti-PDA. However, this is just the response level where interactive effects would be weakest. For more intense stimuli, pigment-state effects on the shape of the LRP have been found, and net pigment shifts affect the strength of a facilitatory effect.

Key words: Invertebrates – Photoreceptors – Visual pigments – Prolonged depolarizing after-potential – Late receptor potential.

Introduction

The discovery of invertebrate photopigment bistability has led to a variety of studies concerning the bistability itself, the responses induced by manipulation of the pigment-state distribution, and the implications of bistable systems for the usual receptor potentials and the underlying transduction process. In this paper, the characteristics of the different responses to stimulation of a photoreceptor containing a bistable-pigment are reviewed in an attempt to answer three questions: Do these responses depend on activation of the same pigment system? Do they have a common ionic

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conductance-change mechanism? Do they have a common transduction process linking the pigment-activation and the conductance change?

The stability of one of the photoproducts of rhodopsin (R) in invertebrate photoreceptors, called metarhodopsin (M) in analogy to vertebrate pigment systems, has been confirmed by the use of spectrophotometry (Hubbard and St. George, 1958; Hamdorf et al., 1968; Stavenga, 1975), electrophysiology (early and late receptor potentials; Nolte and Brown, 1972; Cosens and Briscoe, 1972; Minke et al., 1973a) and psychophysics (Hochstein et al., 1973; Stavenga et al., 1973, 1975; and here reported by Muijser and Stavenga, 1979 and Stavenga, 1979). When rhodopsin and metarhodopsin have absorption spectra with peaks at different wavelengths, or when pH manipulation brings the metarhodopsin to an alkaline state with a different spectrum (Hubbard and St. George, 1958; Lisman and Sheline, 1976) the relative dark distribution of R and M may be experimentally manipulated: Adaptation with light which is favorably absorbed by one state leads to a distribution with relatively little pigment in this state. In fact the distribution has been shown (Hamdorf et al., 1973) to depend on the ratio of the photosensitivities which includes the ratio of the weighting functions (Hochstein et al., 1978) which depend on the transitions among the pigment states and represent the fraction of pigment leaving one state which reaches the other, rather than looping back to the first. The wavelength dependence of the pigment distribution following saturating light is called the saturation spectrum by Hochstein et al. (1978); the Q-curve by Stavenga (1976); and by the new convention will be called the photoequilibrium spectrum (Hillman, 1979). A second spectrum, called the relaxation spectrum, described the wavelength dependence of the rate of approach to this distribution during stimulation. These spectra refer to the pigment system as a whole and not to a single state of the pigment, and thus are useful in determining the pigment-system origin of visual phenomena.

It thus becomes possible to activate differentially and in known quantities the R to M ($R \rightarrow M$) and the M to R ($M \rightarrow R$) chains of the pigment cycle. There is clear evidence for the participation of each of these in the phototransduction process (see below, PDA phenomena). It therefore becomes possible and of interest to study the contributions to various responses by each of the following three types of stimuli:

1. Net $R \rightarrow M$: Stimuli which cause a net shift of pigment from the R to the M state;
2. Net $M \rightarrow R$: Stimuli which cause a net shift of pigment from the M to the R state;
3. Neutral stimuli: Stimuli which cause equal shifts of pigment from R to M and from M to R and thus no *net* transfer at all.

The PDA Phenomena

The most striking phenomenon related to these stimulus types is the prolonged depolarizing after-potential (PDA) already reported in over ten species (*Limulus,* barnacle, fruit fly, dragon fly, blowfly, dronefly, housefly, *Chrysomya,* locust, scallop – where the responses are hyperpolarizing – and even the frog pineal photoreceptor). Figure 1 is reproduced from Minke et al. (1973b), and shows the phenomena in the *Limulus* median eye: A) the dramatic PDA following an $R \rightarrow M$ stimulus;

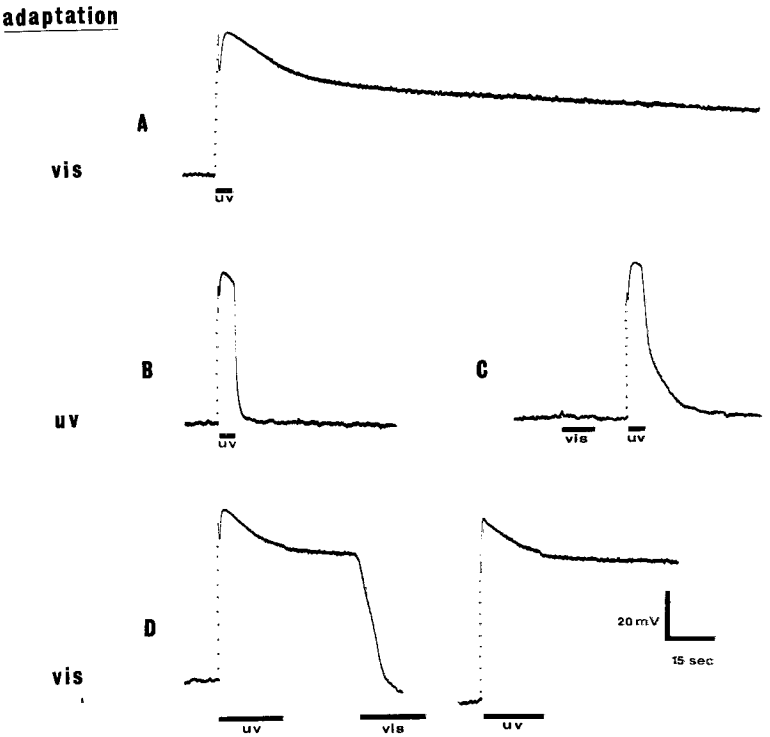

Fig. 1A–D. The prolonged after-effects recorded intracellularly in a *Limulus* median eye UV photoreceptor (from Minke et al., 1973b). **A** The prolonged depolarizing after-potential (PDA) due to a net R → M stimulus (UV following VIS adaptation). **B** The stimulus coincident late receptor potential (LRP) with no PDA, due to a neutral stimulus (no net pigment shift). **C** The anti-PDA effect. The first stimulus (VIS after UV adaptation) produces a net M → R shift and a prolonged after-effect. There is no LRP and no potential accompanying the after-effect. However, the PDA due to a closely-following net R → M stimulus (equivalent to that in **A**) is neutralized and only the LRP is seen. **D** The PDA (produced as in **A**) is depressed by a following net M → R stimulus (VIS following UV adaptation). In this case the anti-PDA effect is neutralized by the PDA which it is in turn neutralizing and another PDA may be immediately induced by a following net R → M stimulus

B) a stimulus-coincident late receptor potential (to be called LRP; see Hillman, 1979) but no PDA, following a neutral stimulus; C) the PDA is neutralized by a previously induced prolonged after-effect called the anti-PDA which is induced by an M → R stimulus; and D) the PDA is depressed by a following M → R stimulus. In D, the anti-PDA is in turn neutralized by the PDA and has no inhibitory effect on the PDA of the following R → M stimulus.

Analysis of the saturation and relaxation spectra of the PDA, PDA depression, and anti-PDA phenomena and the concurrent early receptor potential responses in barnacle receptors has shown quantitatively that the PDA is coupled to R activation causing a net R → M shift and neutralized by M activation causing a net M → R shift (Minke et al., 1978; see also Hamdorf and Razmjoo, 1979). The PDA is unaffected or slightly affected by neutral stimuli or by the state of the unactivated pigment (Hillman et al., 1976)

The LRP Response

The stimulus coincident LRP also depends on R activation (Stratten and Ogden, 1971) but does not require a net R → M tranfer and so seems independent of M activation. In fact the weak criterion action spectrum of the LRP matches the R photosensitivity spectrum and is independent of M activation (Barnes and Goldsmith, 1977; Atzmon et al., 1978, 1979) and even light adaptation depends only on R activation (Strong and Lisman, 1978). However, other data indicate that M activation and possibly presence do play a role in the coupling to the LRP for not so weak stimuli. Hanani and Hillman (1979) report here that in cells which have reduced adaptation effects the degree of facilitation induced by a conditioning stimulus is dependent on the stimulus type and thus on M activation. As to dependence on unactivated pigment molecules, in *Drosophila* (Hamdorf, 1973; Harris et al., 1977), and in several other preparations the LRP sensitivity depends linearly on R population, and the same is true for vitamin A deprived flies (Razmjoo and Hamdorf, 1976). However, using wavelength-adaptation, some flies show a greater than linear dependence on R population (Razmjoo and Hamdorf, 1976), and barnacles a less than linear dependence (Minke, et al., 1974).

Comparison of PDA and LRP Characteristics

These seemingly paradoxical relationships between the characteristics of the PDA and LRP responses lead one to ask whether these two responses depend on the same pigment pool and the same coupling mechanism. Additional characteristics of the PDA and the LRP are now reviewed and summarized, together with those mentioned above, in Table 1.

Ionic Mechanism and Influences. Both the PDA and the LRP have been shown to be the results of light-induced photoreceptor membrane conductance increases (Hochstein et al., 1973; Brown and Cornwall, 1975; Muijser et al., 1975). The main charge carrier is Na^+ but some other ion is also involved since very low external sodium concentrations do not entirely abolish either the LRP or the PDA. In scallop distal photoreceptor cells, the LRP and the PDA are both hyperpolarizing (Cornwall and Gorman, 1976) but the ionic mechanism of only the LRP has been directly studied (increased conductance to K^+ions; Gorman and Cornwall, 1976). Both the LRP and the PDA are depressed and prolonged by increased concentration of Ca^{2+} and Mn^{2+} as reported here by Shaw et al. (1979); and low temperature and high CO_2 and anoxia depress or abolish the LRP and the PDA (Wong et al., 1976; Atzmon, 1978).

Bumps. The LRP is thought to be produced by a sum of discrete voltage fluctuations, called quantum bumps (Fuortes and Yeandle, 1964; Dodge et al., 1968). Minke et al. (1975) and Horridge and Tsukahara (1978) have shown that the voltage rise during the PDA is also associated with quantum bumps. Minke et al. used a mutant with a reduced quantum efficiency and Horridge and Tsukahara looked at the late part of the PDA to measure the voltage fluctuations which are too reduced

Table 1

Characteristic	PDA	LRP
Coupled to R-activation as shown by	Yes	Yes
	Saturation and relaxation spectra	Criterion action spectrum
Neutralized by M-activation	Yes	No
	PDA-depression and anti-PDA	R-spectrum for weak stimuli
		Yes
		Anti-facilitation for intense stimuli
Depends on unactivated R/M	Slight	Species dependent
Due to conductance increase	Yes	Yes
Mainly to Na^+	Yes	Yes
Mainly K^+ in scallop	Yes (PHA)	Yes
Depressed by Ca^{2+}, Mn^{2+}, low temp., high CO_2	Yes	Yes
Is sum of bumps	Yes	Yes
Reduces sensitivity	Yes	Yes
Shows facilitation	Yes	Yes
	One PDA on following; supralinear dependence	In weak cells showing reduced adaptation
Nonlocal facilitation and neutralization	Yes	Yes
Local	Yes	Yes

in size during the early part. Minke et al. also showed that the shot noise variance and autocovariance are similar for the LRP and the PDA in this mutant, indicating both are sums of the same quantum bumps.

Sensitivity. Both the LRP and the PDA reduce the sensitivity of the photoreceptor to additional stimulation. In fact, the reduced sensitivity to test flashes during a PDA

was one of the first characteristics of the PDA studied (Cosens and Briscoe, 1972). The relationship between these effects is discussed here by Hamdorf and Razmjoo (1979).

Facilitation. Both the LRP and the PDA are facilitated by a conditioning stimulus. The PDA following the second of two successive R → M stimuli of equal intensity is greater than the response to the first (Hillman et al., 1976). For cells showing reduced adaptation effects, there is instead a large facilitation effect of conditioning stimuli (Hanani and Hillman, 1976). As mentioned above, Hanani and Hillman (1979) report that this effect is largest for R → M conditioning stimuli.

Local/Nonlocal. In attempting to uncover characteristics of the mechanism underlying a response, an important factor is the degree of spread of the response in the cell. For example, if the receptor pigment is fixed in the membrane and is directly involved in the conductance change mechanism, a single photon activating only one pigment molecule would be expected to increase the conductance at only one site, and there should be no interactions between effects caused by different pigment molecules. In fact, the LRP and the PDA both show facilitation, where activation of one R molecule increases the response to activation of another, and neutralizations where activation of one M molecule reduces the effect of activation of an R molecule. Hillman et al. (1976) found the spread for PDA effects to be at least to next nearest neighboring pigment molecules [though Hamdorf (private communication) did not find this neutralization in *Calliphora*] and Hanani and Hillman's data suggest LRP facilitation over a somewhat larger distance. On the other hand, Almagor et al. (private communication) report that using a spot stimulus, they could show that the PDA activation mechanism does not extend throughout the cell, but is somewhat localized. The same has been shown for the LRP using adaptation as an assay (Fein and Lisman, 1975).

Conclusion

The LRP and the PDA thus have many characteristics in common. These relate mainly to the later stage of the transduction process inducing the conductance change (ionic mechanism, bumps, sensitivity) suggesting that at least this stage of the transduction is common to both responses. In addition, both depend on R activation which is thus the common first stage of transduction. However, the PDA depends on net R → M while M → R neutralizes the PDA. In contrast the LRP seems independent of M → R, depending instead on total R activation rather than on net R → M shift. In fact, weak stimuli which activate M much more than R still show considerable, though sometimes reduced, LRP responses. However, this discrepancy between the PDA and the LRP is found mainly for LRP responses to the weakest stimuli where the least interaction effects would be expected if the interaction range is limited. At higher stimulus intensities, and for cells showing facilitation, this facilitation is reduced for M → R stimuli. How these characteristics are determined by what is presumably an early stage of the transduction process is still unknown.

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