Responses of Barnacle Photoreceptors to High Energy Flashes of Short Duration

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In chromatic adapted barnacle median and lateral photoreceptors the two stable states of the photopigment (rhodopsin R and metarhodopsin M) were interconverted with intense, colored light flashes of 1 ms duration. Only after conversion of the red adapted photoreceptor in K^+-Ringer solution with an intense flash the negative early receptor potential, ERP (of R) gradually appeared detected with an indicator flash. For the opposite conversion (blue adapted, $R \rightarrow M$) the gradual appearence of the positive ERP (M) was not measurable in the same time span. In artificial seawater all flash stimuli yielded — irrespective of color — the transient component of the late receptor potential (LRP). ERP results for the lateral photoreceptor are discussed in view of an existing kinetic model and an attempt is made to give an explanation which covers the new LRP transient *and* ERP results for both types of photoreceptor (appendix).

Introduction

The electrical response of photoreceptor cells to physiological light stimulation is the receptor potential (late receptor potential, LRP). During the receptor potential the membrane conductance and the potential drop across the membrane change. The so called transient component at the beginning of the LRP has a higher amplitude than the following plateau phase. The LRP is initiated (and/or controlled?) by photochemical reactions of photoconvertible pigments which change their absorption as a consequence of light absorption.

Among invertebrate photoreceptors the photoconvertible pigment system has two stable states [1, 2]. For the barnacle the two states were attributed to rhodopsin (R) and metarhodopsin (M) [2]. For some invertebrate photoreceptors, *i. e.* the limulus ventral photoreceptor, each pigment conversion gives an ERP of certain polarity [2, 3] and has a characteristic absorption spectrum. For the barnacle photoreceptors the action spectrum of the negative *i. e.* hyperpolarizing ERP (*i. e.* for the conversion $R \rightarrow M$) has $\lambda_{max} = 532$ nm [2]. Pigment conversions which are elicited by light steps in chromatic adapted barnacle photoreceptors yield ERP's followed by the depolarizing *i. e.* positive LRP's [4, 5] with the two components, the transient and plateau phase. After the cessation of a stimulus which converts most of R into M the depolarization persists; this is the *p*rolonged *d*epolarizing *a*fterpotential, PDA (the reverse conversion $M \rightarrow R$ gives PDA depression, the anti-PDA [4]). The direction of the pigment conversion therefore influences both, ERP's and — after the plateau phase of the LRP — the PDA. Some barnacle photoreceptors loose during the preparation in artificial seawater (ASW) the resting potential. Cells which exhibited this "spontaneous LRP disappearence" [2] and which had a cell resistance $1/50 - 1/100$ of the normal value served to establish a detailed kinetic model for the pigment transition with light pulses leading to complete conversions within about 60 ms [6]. The ERP's served as indicator for the various pigment species.

The effect of the pigment conversions $M \rightarrow R$, $R \rightarrow M$ on the LRP transient in ASW has not yet been studied systematically even though it has been shown that the LRP transient can be elicited for both directions of pigment conversion [7].

It is the aim of this note to present results which are new (especially compared to previously published data [6]) in that they: a) are obtained with very short flashes (isomerization in 1 ms compared to 60 ms [6]), b) show reversible LRP-suppression (compared to irreversible LRP disappearence [2, 6]) and c) also cover results from the barnacle median photoreceptor (which was not studied in the mentioned publication [6]). They will be briefly discussed in view of an existing kinetic model for light induced pigment transitions [6]. To cover most of the phenomena which lie beyond the model [6] (influence of membrane voltage and comparison of the two photoreceptor types) a membrane model is offered (appendix) which accounts for the recently reported photostable pigment [8, 9] and the specific electric membrane properties of the two types of barnacle photoreceptors [5].

Methods

The preparation of the rock barnacle, *Balanus eburneus*, the extracellular air gap recording technique of the receptor potentials and the optical system for adaptation and stimulation of the photoreceptors have been described in detail previously [5].

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Briefly, the electrolyte was artificial seawater (ASW [5]) or Balanus Ringer [10] in which Na^+ was exchanged by K^+. The lateral photoreceptors faced with the tapetum the stimulating optical system, the adapting light entered from the opposite side (from below). The resistance of the nerve was constant for one experiment and had a range of 0.5 – 2 MOhm.

The *optical system* consisted of two perpendicularly arranged channels which were part of a binocular Leitz Orthoplan microscope with a $\times 10$ objective.

The *stimulating light* consisted of two Rollei E20 C photoflashes (flash duration 1 ms) with provision for interposing optical filters (red, Schott OG 570: $\lambda \geqq 570$ nm and blue interference filters: Schott DT blue with $\lambda_{max} = 440$ nm and bandwidth $\Delta\lambda \approx 100$ nm or Balzers K2 with $\lambda_{max} = 455$ nm, $\Delta\lambda_{max} \approx 45$ nm). The *energy* of the *white flash stimuli* was measured by attenuating the stimulus by 5 log units giving a response amplitude 30 – 70% of the saturation amplitude. From this it can be concluded [11] that each unattenuated white flash contained at least about 10^{17} absorbable photon/cm².

The *adapting light* was the tungsten filament lamp of the condensor system of the microscope with the provision of placing the optical filters in the light path. The effective light intensity depended somewhat on the orientation of the preparation (with the rests of adhering tissue) but a pigment conversion was completed within 1 – 10 seconds and gave receptor potentials $\geqq 80\%$ of maximal value (near saturation). The effective quantum flux density of the colored adapting ligth is therefore with published sensitivity data [11] calculated to about 5×10^{14} photon/cm² s.

Results

The measurements with the PDA – anti-PDA phenomenon in seawater with *colored flashes* for both photoreceptors gave qualitatively the same results as reported in the literature [4, 7, 12]. The *LRP-transient component* could be elicited with 1 ms flashes of any color and it was independent of the state of adaptation. After a flash induced LRP transient a second transient could only be evoked after a certain delay. This recovery time differed for the two types of photoreceptor by approximately a factor of two: 117 ± 15 ms were needed for the median (s.d. $n = 5$) and 164 ± 18 ms for the lateral photoreceptor (s.d. $n = 5$) to reach 64% of the maximal amplitude, see also Figs 1 A, B and 2 A.

The *early response* of the photoreceptors to *one saturating flash*, the ERP, was in seawater difficult to measure because the decay phase of the ERP coincides with the beginning of the LRP. Cells which

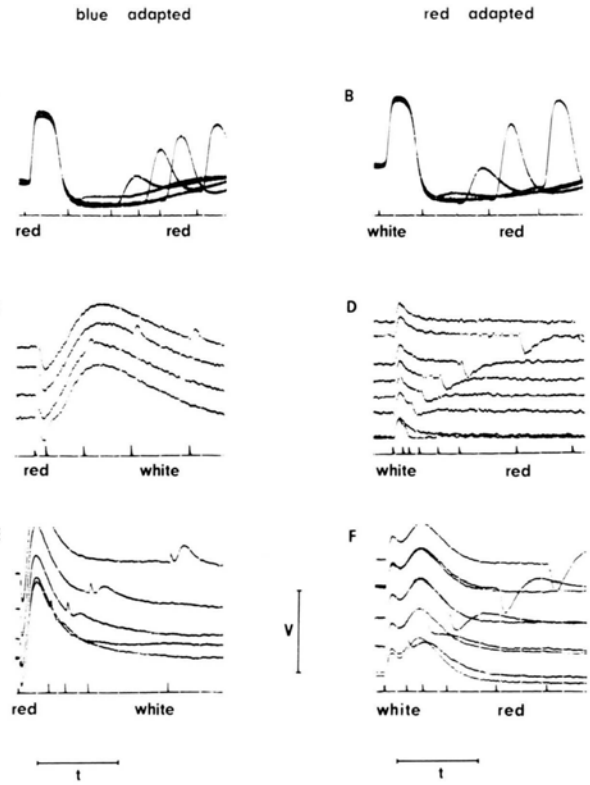

Fig. 1. Examples for early and late receptor potentials of barnacle photoreceptors induced by two subsequent colored flashes with varying delay. Background light was in the first column A—E blue and in the second B—F red. Within each row A,B; C,D and E,F results are from one photoreceptor; the results from the different rows, however are from different animals. The color of the first converting flash and the second indicator flash is indicated below the recordings. For A and B (one median photoreceptor) the results were almost the same if the second flashes were white instead of red. C—F are records of lateral photoreceptors. A and B: ASW-electrolyte, C—F K^+-Ringer solution, see text. Marks at the bottom of each group of records represent photodiode records of light flashes. A, B, D (lowest trace) and F lower four traces: superimposed with and without second flash. Measuring temperature 22 ± 2 °C. Note the gradual appearance of the second ERP in D and F, see also Fig. 2 B, dots. Also note that the 2nd ERP's in C and E are in amplitude and time course not characteristic of the positive ERP (as seen in the records of D and F, beginning). Calibration bars: V: A, B, C and D = 2 mV and E, F = 4 mV; t: A, B and F = 80 ms; C and D = 40 ms and E = 200 ms.

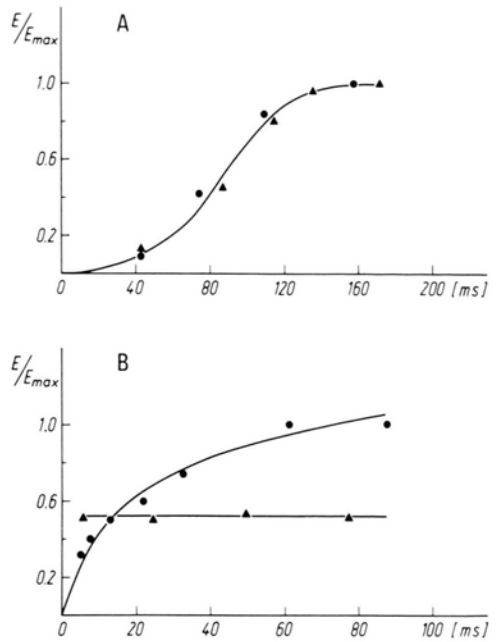

Fig. 2. Response amplitude, normalized to maximal amplitude, as a function of the delay between conversion (first) and indicator (second) flash (both of saturating energy).
A: median photoreceptor, LRP data of Fig. 1 A (triangles) and Fig. 1 B (dots).
B: lateral photoreceptor, ERP data of Fig. 1 C (triangles) and Fig. 1 D (dots).

gave the LRP were therefore depolarized with K^+-Ringer solution in order to suppress the LRP (even after 3 – 4 electrolyte exchanges the lateral photoreceptor had still 1 – 10% of the maximal LRP amplitude in seawater Fig. 1 C, E and F, probably because the corneal capsule hinders the access of electrolyte). In a few cases the median photoreceptor gave in ASW no LRP, "spontaneous LRP dissappearance". Such cells gave, however, qualitatively the same conversion kinetics as cells which had been bathed long enough in K^+-Ringer to give no more LRP's; *i. e.* the kinetics was similar as that in Fig. 1 D.

The time course and polarity of *single* flash induced *ERP's* of both types of photoreceptor were measured in accord with previously reported measurements [2, 5, 6]: After the cell was *red adapted* either with steady red light or with a red flash and a subsequent dark period of several seconds a positive ERP (of M) was observed with blue or white flashes (initial responses in Fig. 1 D and 1 F), whereas a red flash gave under these adaptation conditions no or only a very small negative response. For *blue adaption* (steady ligth or flash followed by dark) red flashes always elicited a negative ERP (of R, initial peaks in Fig. 1 C and 1 E). With blue or white flashes, *i. e.* with flashes of the same color as the adapting light one could also obtain negative responses of high amplitude. White and blue flashes can therefore elicit ERP's of either polarity depending of the state of adaptation whereas red flashes only yield negative ERP's.

For the lateral photoreceptor the amplitude of the positive and negative ERP were about the same. The median photoreceptor had a smaller maximal negative amplitude, the ratio (negative/positive) being 0.56 ± 0.15 (s.d. $n = 8$).

The rapid *pigment conversion* from one adaptation state to the other was followed with a *sequence of two colored flashes*. One flash, the first, served to convert the pigment, the other, the second one (indicator flash), to measure (by means of the resulting ERP) the rate of the pigment conversion. Whenever there was in ASW a transient of high amplitude (as in Fig. 1 A and B) the ERP due to the second flash was not measurable during the transient but began to appear after the transient had gone through the maximal amplitude. The ERP's due to the first flash are in Fig. 1 A and B not resolved. To suppress the LRP transient reversibly the bulk of the two flash experiments were therefore carried out in K^+-Ringer solution, Fig. 1 C – 1 F.

The usual positive ERP appears for both types of photoreceptor if the conversion flash (1st, white) is superimposed on *red background light*. The indicator flash (2nd, red) gives, depending on the delay time, the expected negative ERP. The recovery time of ERP amplitude (to 64% of the maximal amplitude) was about 24 ms for the lateral photoreceptor in Fig. 1 D, see also Fig. 2 B. In cells with rest-LRP (Fig. 1 F) the negative ERP appeared – similar to Fig. 1 D – also gradually. The recovery time constant was somewhat longer than in cells with no rest-LRP. The recovery time for the median photoreceptor was shorter than for the lateral photoreceptor; usually by a factor near 0.5.

The opposite conversion, *i. e.* of photoreceptors which were adapted with *blue background light* gave with the red conversion flash the expected negative ERP of the blue adapted pigment (R), Fig. 1 C and 1 E, but with the indicator flash (white or blue, high energy) one did, up to delay times of 400 ms,

not observe the gradual appearence of the positive ERP in the usual amplitude and shape, Fig. 1 C, E and 2 B, triangles.

The chromatic adaptation with steady light or with high energy flashes (without longer dark periods) apparently does not lead to the same ERP's. For example, red adaptation with low quantum flux densities gives an ERP of full amplitude (first responses in Fig. 1 D and 1 F) but only a small amplitude is elicited by a red flash (Fig. 1 C and 1 E). The two types of pigment conversions by high quantum flux density flashes therefore exhibit asymmetry in amplitude and time course of the recovery of the ERP's, see Fig. 2 B,

Discussion

The data agree to some extent with the reported kinetic model [6]:

1) The transition of the blue adapted state, R (with negative ERP, A in the model [6]) to the red adapted state, M (with positive ERP, D in the model [6]), *i. e.* A → D is considerably faster than the reverse transition, D → A, Figs 1 C – F, 2 B, even though the absolute transition time for D → A is for completely suppressed LRP shorter, 24 ms in Fig. 1 D compared to about 85 ms for the cells with complete LRP disappearence [6]. The positive ERP's which are generated by conversion with intense red flashes (A → D) yield in the first 400 ms only 0.2 – 0.5 of the maximal amplitude, Fig. 1 C, E whereas the model [6] predicts within such a time span a full ERP amplitude. A membrane model is proposed in the appendix to explain this and the observed difference between the two types of barnacle photoreceptors and the influence of membrane voltage on the ERP's. This model also explains that the sensitivity of D is higher than that of A, a ratio 4 : 1 for D to A was reported [6].

Appendix, Membrane Model

In barnacle photoreceptors the dual function of the visual pigment rhodopsin in undergoing photochemical reactions and in contributing to the cell's electrical properties [5] suggest to propose a model in which the recently observed photostable pigment [8, 9]) is also assumed to be incorporated into the cell membrane system and participates – like the convertible pigment system – with a dual function, a photochemical one by being capable of transferring part of the absorbed energy [8, 13] to the blue absorbing pigment species of the convertible pigment system (M, D [6]) and an electrical one by adding more leaks to the cell membrane system. The parameter which causes a coupling of the two types of function might be membrane voltage, current or ionic milieu.

The following observations can be explained:

1) The asymmetry of the conversion kinetics between A → D Fig. 1 C, E and D → A, Fig. 1 D, F because one type of conversion involves only the photoconvertible pigment system (R → M, Fig. 1 C, E) whereas the opposite conversion (M → R Fig. 1 D, F) makes use of both types of membrane bound pigment systems, photoconvertible *and* photostable.

2) The influence of membrane voltage on conversion kinetics Fig. 1 D, F because the same membrane process which prevents ERP generation during the LRP transient also becomes operative by depolarization of the cells with K^+-Ringer solution and

3) the difference between the two types of photoreceptor a) in the ratio of positive/negative ERP amplitude (median larger) and b) conversion kinetics (median faster) because the median photoreceptor is assumed to contain relatively more photostable pigment than the lateral photoreceptor. This photoreceptor can with this additional blue absorbing membrane utilize more effectively the energy of blue photons (relatively more positive ERP amplitude) and, because of the additional leaks, accomplish a faster conversion.

Still unknown is the mechanism of energy transfer between the pigment systems. The observed strong temperature dependence of the conversion reactions [6] favors a mechanism in which only a small fraction of a photon's energy is transferred (exciton coupling?). Also not clear is the physiological function of the photostable pigment. It could support the rhodopsin regeneration for the exitation process (LRP results of this note). This function would especially be useful for the fast responding median photoreceptor for the quick restoration of exitable rhodopsin. Such an advantage of rapid restoration of exitability would with the proposed model also have disadvantages, some of which are known: The

median photoreceptor has a more leaky membrane system [5], gives a smaller steady state response amplitude [14] and is less sensitive than the lateral photoreceptor [15]. The discussion showed that the existing kinetic model for the pigment transitions [6] needs therefore only to be extended to cope with the proposed additional energy supply and the membrane nature of the pigments; the intermediates of the model [6] can be the same.

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Erratum

F. Bordin, F. Baccichetti, and F. Carlassare, 4,5-Dimethylangelicin, a New Very Active Monofunctional Furocoumarin, Z. Naturforsch. **33 c,** 296 (1978). Page 296, 3rd line should be read: F. Bordin, F. Baccichetti, and F. Carlassare.

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