The Sensitivity of the Ventral Nerve Photoreceptor of *Limulus* Recovers after Light Adaptation in Two Phases of Dark Adaptation

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The time course of the recovery of the sensitivity of the *Limulus* ventral nerve photoreceptor was measured during dark adaptation following light adaptation by a bright 1 or 5 s illumination. The stimulus intensity I_{CR} of a 300 µs light flash evoking a response of criterion amplitude (receptor potential or receptor current under voltage clamp conditions) was used as measure of sensitivity.

The time course of dark adaptation shows two phases with time constants in the range of 5–9 s and 300–500 s (15 °C). Only the first of the two phases is significantly changed when the extracellular Ca^{2+}-concentration is varied.

The power function $I_{CR} = a \cdot I_o \cdot t_{DA}^{-b}$ gives a good data fit for each of the two phases of dark adaptation. In the first phase the factor a_1 and the exponent b_1 are decreased when the external calcium is lowered from 10 mmol/l to 250 µmol/l. Conversely a_1 and b_1 are increased when the Ca^{2+}-concentration is raised to 40 mmol/l. For the second phase neither a_2 nor b_2 is changed significantly upon the changes in calcium concentration in the same experiments.

The two phases of dark adaptation reflect the behaviour of the two components C_1 and C_2 of the electrical light response (receptor potential or receptor current). Under the conditions described here C_1 determines the size of the light response during the first phase of dark adaptation whereas C_2 mainly influences the size of the response during the second phase.

Interpretation: The fast first phase of dark adaptation is determined by the change in intracellular Ca^{2+}-concentration. The slower second phase of dark adaptation is not primarily calcium-controlled.

Introduction

A light stimulus causes an electrical light response and a transient desensitization of the photoreceptor cell. The desensitization (light adaptation) of a photoreceptor is due to changes controlling various steps in the transduction process [1, 2]. As shown by J. Lisman, J. E. Brown, A. Fein and us (see [3–6]) changes in the intracellular Ca^{2+}-concentration control the sensitivity of the *Limulus* photoreceptor cell in adaptation. However, this is not the only mechanism which controls the sensitivity of the photoreceptors of invertebrates in light and dark adaptation [6–8]. *E.g.* very weak light adaptation, which is weak enough that individual bumps can be measured, is not substantially calcium dependent [9].

Different processes controlling the sensitivity may become recognizable during dark adaptation in the time course of the recovery of the sensitivity following desensitization. The time course of dark adaptation following desensitization can be tracked by measurement of the light response evoked by a constant test flash following a conditioning illumination after various adaptation times t_{DA} (constant stimulus curve). Constant stimulus curves are often measured

Abbreviations: PMP, prestimulus membrane potential; ReP, receptor potential; h_{max} [mV], peak amplitude of ReP; t_{lat} [ms], latent period (from stimulus begin until first measurable increase of response); t_{max} [ms], time-to-peak from stimulus begin; ReC, receptor current; J_{max} [nA], peak amplitude of light-induced current signal; U_{CR} [mV], criterion height of voltage response; U_{Cl} [mV], clamped membrane voltage; J_{CR} [nA], criterion height of current response; I, light intensity; I_o, maximum light intensity available; LA, conditioning (light-adapting) stimulus; t_{DA}, dark adaptation time (from the end of the conditioning (light-adapting) illumination to the beginning of the test stimulus); $[Ca^{2+}]_e$, extracellular calcium ion concentration; $[Ca^{2+}]_i$ intracellular calcium ion concentration; C_1, C_2, components of light response; $s_{\bar{x}}$, standard error of the mean; n, number of experiments; PS, physiological saline.

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because they are relatively easy to obtain experimentally. But they are not very informative, because they contain the intensity dependence of the response, which is not linear. Nevertheless two phases of dark adaptation could be demonstrated in *Limulus* photoreceptors by such measurements [7, 10].

In the experiments presented here the sensitivity of the cell was determined in dependence of the time t_{DA} of dark adaptation following a conditioning illumination. Sensitivity was defined as reciprocal intensity of the light stimulus which is needed to evoke a response of a certain, arbitrarily chosen, criterion amplitude. Sensitivity curves for criterion responses allow to characterize directly the change in sensitivity of a receptor. The curves will differ according to the criterion chosen: This may be the membrane voltage signal (receptor potential) which is the physiologically meaningful signal used and processed in the animal by the synapses which connect the visual cells with the following nerve cells. The membrane current signal (receptor current), measured under voltage clamp conditions, gives more direct information about the transduction process. Since the shape of the receptor current signal changes with adaptation, responses of the same height at different times of dark adaptation have different time course. Therefore the current-time integral of the light response is also a sensible measure. First experiments using the criterion response method to study dark adaptation at the *Limulus* lateral eye were already performed by Hartline [11] and later on by Benolken [12]; Fuortes and Hodgkin [13] investigated the adaptation process of *Limulus* lateral eye in relation to the photochemical cycle.

The investigations reported in this paper were aimed at determining the time course of sensitivity recovery during dark adaptation and describing it quantitatively. In addition we studied how dark adaptation depended on the Ca^{2+}-concentration of the superfusate. Two phases in the recovery of the sensitivity were observed: One during the first 10 to 30 s, which was followed by a second phase lasting up to 10–20 min. Only the first phase depended significantly on the external Ca^{2+}-concentration, which was raised from 10 to 40 mmol/l or lowered to 250 μmol/l. The results indicate that the first phase of dark adaptation is controlled by the intracellular Ca^{2+}-concentration. The mechanism which controls the second phase of dark adaptation is not yet known.

Materials and Methods

Limulus ventral nerves were excised, dissected and desheathed under dim red light and bathed in 0.5% pronase solution for 45 s. After this dessection the ventral nerve was mounted in a plexiglass chamber and fixed on a Sylgard bedding. The nerve was continuously (1 ml/min) superfused by physiological saline (composition: 481 mmol/l NaCl, 10 mmol/l KCl, 10 mmol/l $CaCl_2$, 25 mmol/l $MgCl_2$, 30 mmol/l $MgSO_4$, 10 mmol/l Hepes buffered with 5 mmol/l NaOH (2n); pH: 7.5) or test salines of changed Ca^{2+}-concentration. 1) low $[Ca^{2+}]$ (0.25 mmol/l $CaCl_2$ instead of 10 mmol/l $CaCl_2$ in the physiological saline); 2) high $[Ca^{2+}]$ (40 mmol/l $CaCl_2$). The temperature was 15 °C or 21 °C. The osmotic pressure of the physiological (reference) saline was adjusted to that of the test saline if necessary by addition of sucrose (for more details see [14, 15]).

The receptor potential was recorded intracellularly in a standard way [14]. The voltage clamp procedure used is described elsewhere [16]. The conditioning, light-adapting stimulus was a 1 or 5 s illumination which was delivered by a xenon lamp; the intensity corresponded to 4.4×10^{16} photons $cm^{-2}s^{-1}$. Light of λ_{max} 543 nm (half width 50 nm) was used for conditioning illumination and test stimuli. The test flash was a 300 μs almost square flash which was delivered by a flash lamp (Metz Mecablitz, 60 CTl) and corresponded to 1.4×10^{14} photons cm^{-2}. This maximal test flash intensity I_o could be attenuated by neutral density filters ($I/I_o = 1/2$ f; f = filter = 0, 1, 2, 3, ...). In the set-up used for the voltage clamp experiments a mercury vapour lamp was used as light source for the adapting illumination (2 s, ca. 10^{15} photons $cm^{-2}s^{-1}$) and the test stimulus was a 300 μs flash of the Metz Mecablitz flash lamp which corresponded to ca. 2.6×10^{14} photons cm^{-2}. Otherwise see figure legends.

Experimental procedure

The time course of the increase of sensitivity was determined by a stimulus sequence consisting of a conditioning (light-adapting) stimulus followed by a test flash which was applied after a variable delay time t_{DA}. The sequence was repeated periodically after a cycle time of 2 min (or 90 s in the voltage clamp experiments).

The time t_{DA} for a certain constant test flash intensity was varied from cycle to cycle to determine the

dark adaptation time after which the particular test flash evoked a response of a certain criterion amplitude (U_{CR} of the receptor potential or J_{CR} of the receptor current, see Figs. 1a and b). Then the test flash intensity was lowered. For each stimulus intensity the dark adaptation time t_{DA} for a response of criterion amplitude was determined. The criterion amplitude was chosen to be half that of the response evoked by the strong conditioning (light-adapting) stimulus. As tested by families of constant stimulus curves, the results were not substantially different for different criterion heights as long as these were between 20% and 80% of response amplitude saturation (see Fig. 2). The time t_{DA} which was necessary to evoke a response of criterion height by a certain stimulus intensity was determined by linear interpolation between measured points as shown in Fig. 2.

For very weak test stimulus intensities the dark adaptation time t_{DA} needed to reach the criterion response height was longer than 2 min. In these cases test flashes were delivered every minute after light adaptation until the criterion response amplitude was reached; since these repeated test stimuli were very weak they did not substantially change the state of adaptation. After long dark adaptation times several conditioning (light-adapting) stimuli were administered successively in the 2 min cycle (see Fig. 1b); this was necessary to obtain the same level of adapta-

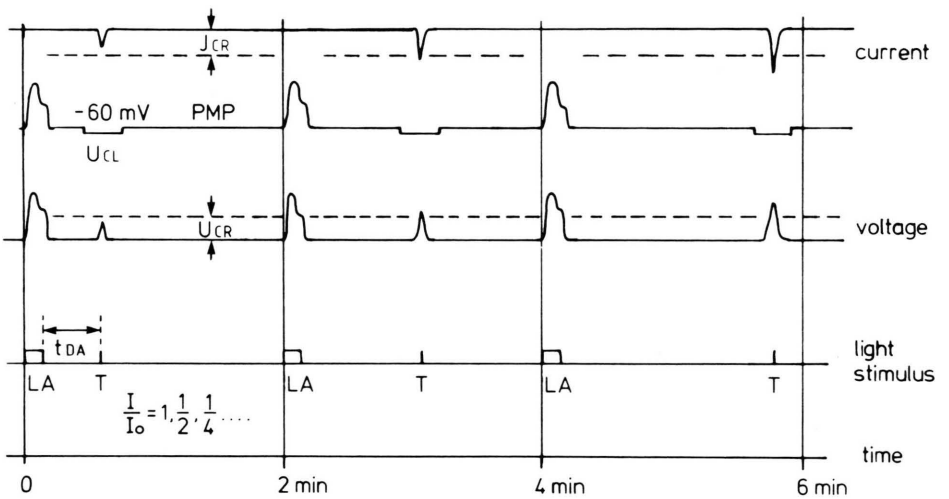


Fig. 1a. Time regime of current measurement (J), voltage measurement (U) and stimulus sequence (lower trace). U_{Cl} clamped membrane voltage, U_{CR} receptor voltage of criterion height, I_{CR} receptor current of criterion height, LA conditioning (light-adapting) stimulus (1 or 5 s, λ_{max} 543 nm, intensity equivalent to 4.4×10^{16} photons $cm^{-2}s^{-1}$). T test flash of variable light intensities applied at different times of dark adaptation (t_{DA}); for details see procedure.

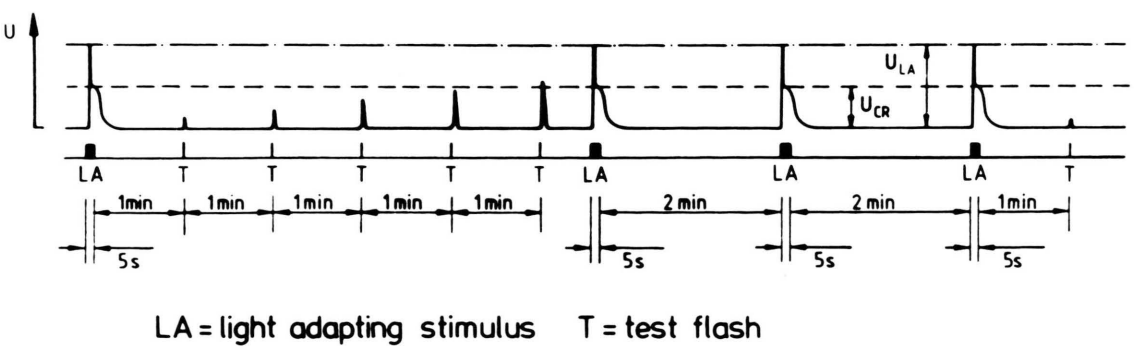


Fig. 1b. Stimulus regime for $t_{DA} > 2$ min.

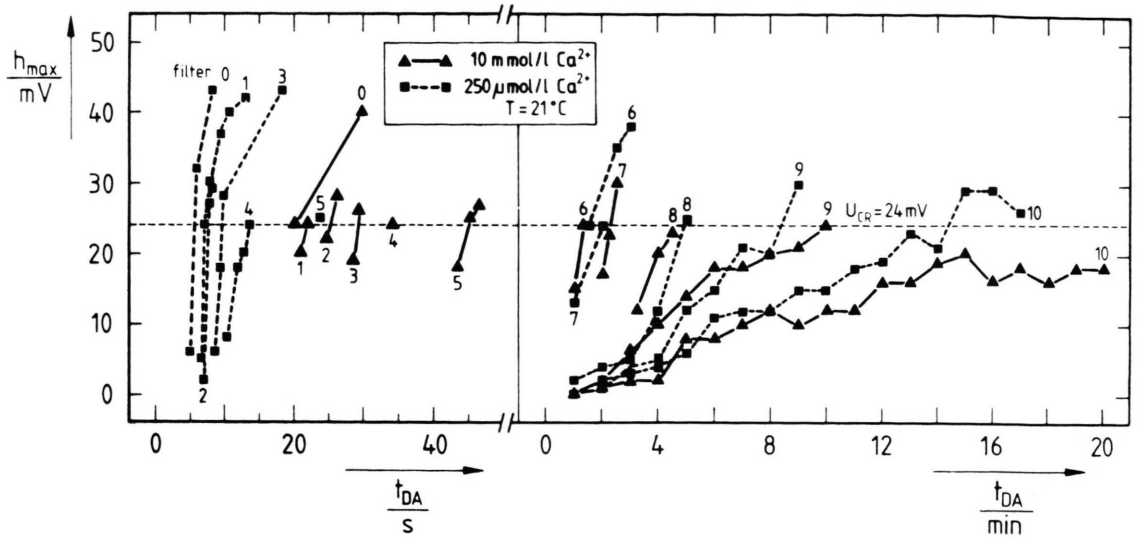


Fig. 2. Response amplitude as function of time of dark adaptation t_{DA}. Constant stimulus curves in physiological saline (10 mmol/l Ca^{2+}) and in low Ca^{2+}-saline (250 µmol/l Ca^{2+}). The dotted line indicates the criterion response amplitude U_{CR} 24 mV. Same experiment as shown in Fig. 6. (Li 5)

tion as before. The scheme of the stimulating and recording regime applied in the experiments is illustrated schematically in Fig. 1a, b.

Evaluation

The following parameters of the receptor potential were evaluated: h_{max} [mV], peak amplitude of ReP; t_{lat} [ms], latent-period; t_{max} [ms], time-to-peak (see abbreviations).

In the voltage clamp experiments the peak amplitude of the light-induced current signal J_{max} [nA] was measured.

The light intensity evoking a response of criterion amplitude (*i.e.* the reciprocal sensitivity) independence of dark adaptation time t_{DA} was fitted by two exponential functions

$$I_{CR} = \alpha \cdot I_o \cdot e^{-\frac{t_{DA}}{\tau}} \quad (1)$$

or by two power functions

$$I_{CR} = a \cdot I_o \cdot {t_{DA}}^{-b} \quad (2)$$

I intensity; I_o maximal intensity used. The factor a characterizes the reciprocal sensitivity for t_{DA} = 1 s. It varies greatly from experiment to experiment. The exponent b characterizes the slope.

The power functions are used in the following because they gave the better data fits – (that means higher correlation coefficients, r^2-values) – to the measured data as compared to the exponential functions.

Results

1. Criterion response of receptor current and receptor voltage signal

Depending on the state of adaptation, and thus on t_{DA}, and on the intensity of the test flash, the shape of the responses of the same criterion height varied (see Figs. 3 and 8) and showed the two components C_1 and C_2 with varying distinctness (see below, compare also [17]).

2. Sensitivity curve for criterion response

In the double logarithmic plot of a sensitivity curve to evoke a receptor potential of criterion amplitude (Fig. 4) two different slopes are observed, the first one being much steeper, *i.e.* with a greater negative exponent b of the power function (equation (2)), than the second one. That means that the sensitivity recovers much faster in the first phase of dark adaptation than in the second phase. The break between the two slopes occurred mostly between light intensities $I/I_o = 10^{-1}$ and 10^{-2}. On the average b_1 was 3.5 (± 0.4; n = 16), b_2 for the second slope was 1.0 (± 0.1; n = 13). The factors a_1 and a_2 (equation (2))

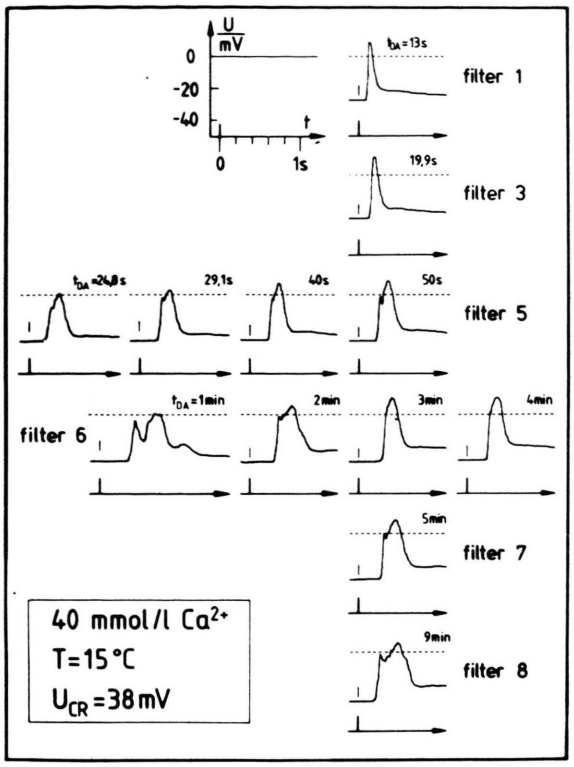


Fig. 3. Receptor potentials evoked by various stimuli (light intensities filter 1–8, vertical columns) in saline with 40 mmol/l Ca^{2+}. Dark adaptation time t_{DA} increased from 13 s to 9 min (horizontal lines). The amplitude of each light response is close to the criterion height of 38 mV. (Cl 4A)

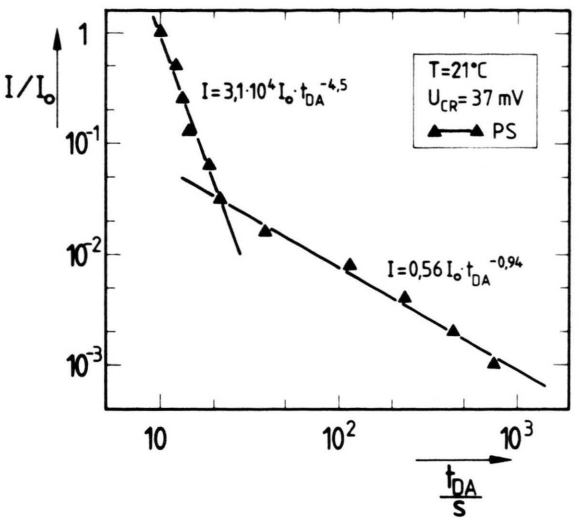


Fig. 4. Time course of sensitivity recovery during dark adaptation monitored by the normalized intensity I/I_o of the test stimulus evoking a criterion amplitude U_{CR} 37 mV of the light-evoked receptor potential. In the double logarithmic plot the dark adaptation shows two phases, an initial fast one followed by a second slower phase. (Li 1)

vary greatly from experiment to experiment. Fitted by exponential functions (equation (1)) the time constants of the two phases range between 5 and 9 s (τ_1) and between 300 and 500 s (τ_2).

Measuring receptor *current* responses of criterion amplitude under voltage clamp conditions we also found two different adaptation phases (Fig. 5). This shows that the occurrence of the two different phases of dark adaptation is not due to properties of voltage-sensitive membrane conductances participating in the light response, since these do not contribute to the receptor current under voltage clamp conditions. Both phases of dark adaptation therefore are based on a light-induced conductance increase of the photosensory receptor membrane.

In some experiments receptor currents and receptor potentials were recorded alternatingly. They showed that the stimulus intensity evoking a response of criterion amplitude decreased with increas-

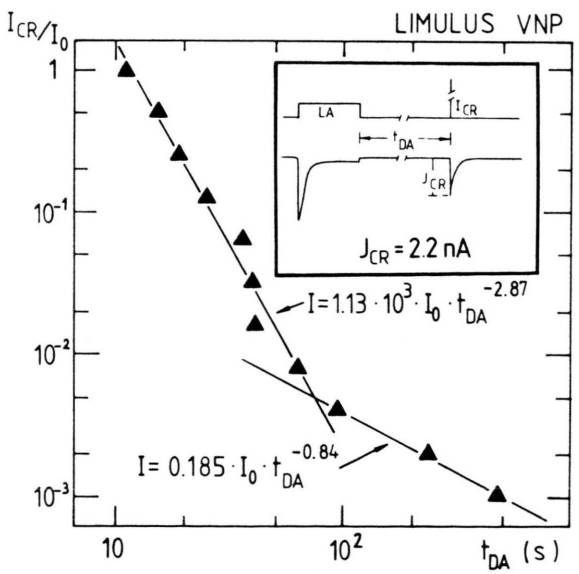


Fig. 5. Time course of sensitivity recovery during dark adaptation monitored by the normalized intensity I/I_o of the test stimulus that evokes a criterion amplitude J_{CR} 2.2 nA of the light-evoked receptor current (voltage clamped, double logarithmic plot). 2 s light-adapting illumination: I_{LA} ca. 10^{15} (550 nm) photons $cm^{-2}s^{-1}$. 300 µsec test stimulus, I_o ca. 2.6×10^{14} (550 nm) photons cm^{-2}, 17 °C.

ing dark adaptation times t_{DA} to a different extent for the receptor current as compared to the receptor potential (Fig. 8). The main reason is presumably the non-linearity of the rise of the receptor potential with t_{DA} or stimulus intensity. The time t_{DA} of the break in the slope of the sensitivity recovery is different for receptor current and receptor potential. At the same state of adaptation (see below) the two components contribute to different degrees to the light-induced receptor current signal as compared to the voltage signal.

3. *The influence of extracellular calcium concentration*

Lowering the extracellular Ca^{2+}-concentration from 10 mmol/l to 250 μmol/l influences the slope of the first phase of dark adaptation strongly, but the second phase not significantly (see Fig. 6).

On the average the exponent b_1 is changed from 3.5 ± 0.4 ($n = 16$) to 1.7 ± 0.5 ($n = 6$), b_2 from 1.0 ± 0.1 ($n = 13$) to 1.2 ± 0.2 ($n = 4$). The sensitivity of the photoreceptor cell is raised (the factor a is decreased) in low external Ca^{2+}-concentration during the first phase of dark adaptation; shorter dark adaptation times t_{DA} are required to evoke a response of criterion amplitude.

Raising the extracellular Ca^{2+}-concentration from 10 mmol/l to 40 mmol/l has the opposite effect on the first phase of the dark adaptation as compared to lowering the external Ca^{2+}-concentration (see Fig. 7). The slope of the first phase characterized by the negative exponent b_1 is raised, on the average from 3.5 to 4.7 ± 0.5 ($n = 3$). The slope of the second phase is not changed significantly compared to the value obtained in physiological saline. In contrast to low external Ca^{2+}-concentration the sensitivity of the photoreceptor cell after 1 s of dark adaptation is lowered (a_2 is enlarged) in high extracellular Ca^{2+}-concentration during the first phase, *i.e.* longer dark adaptation times are required to evoke a response of criterion amplitude.

4. *Two components*

Both in receptor current and receptor potential two components C_1 and C_2 of the light response (see [17]) could be observed. They depended strongly on stimulus intensity and dark adaptation (see Figs. 8 and 9). When the components in the current and voltage response of one cell are compared, C_2 be-

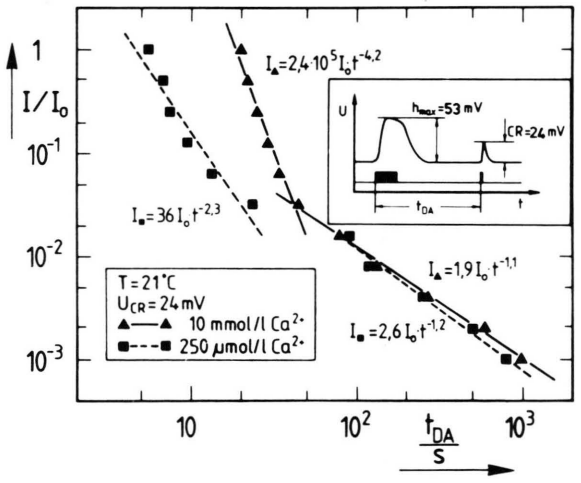


Fig. 6. Time course of sensitivity recovery during dark adaptation monitored by the normalized intensity I/I_o of the test stimulus evoking a criterion amplitude U_{CR} 24 mV of the light-evoked receptor potential. Criterion response curve in PS (10 mmol/l Ca^{2+}) and in low Ca^{2+} (250 μmol/l Ca^{2+}); double logarithmic plot of normalized stimulus intensity I/I_o versus dark adaptation time t_{DA}. Only the first phase of dark adaptation is markedly influenced by the lowering of the extracellular calcium concentration. (Li 5)

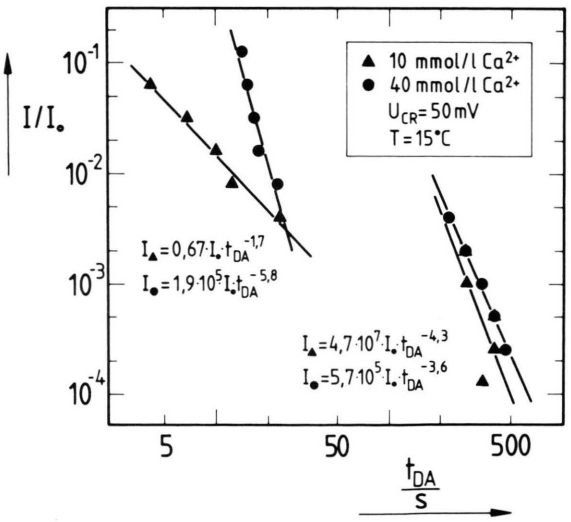


Fig. 7. Time course of sensitivity recovery during dark adaptation monitored by the normalized intensity I/I_o of the test stimulus evoking a criterion amplitude U_{CR} 50 mV of the light-evoked receptor potential. Criterion response curve in PS (10 mmol/l Ca^{2+}) and in high Ca^{2+} (40 mmol/l Ca^{2+}); double logarithmic plot of normalized stimulus intensity I/I_o versus dark adaptation time t_{DA}. Only the first phase of dark adaptation is markedly influenced by the raised extracellular calcium concentration. (Cl 6A)

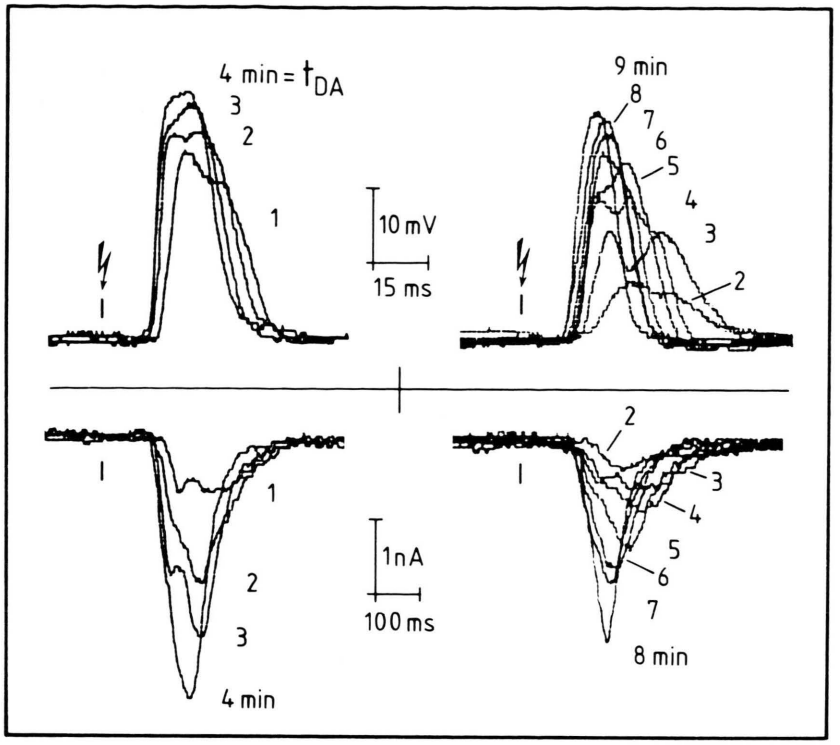
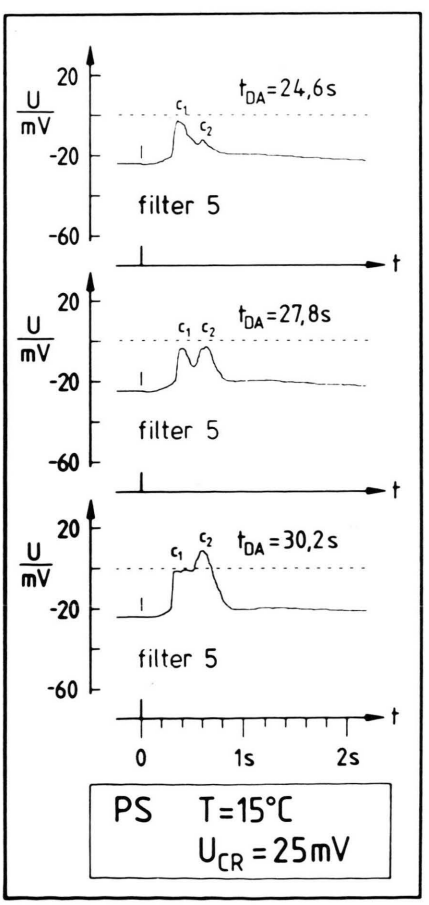


Fig. 8. Light-induced membrane voltage signals and light-induced membrane current signals under voltage clamp, demonstrating the two components C_1 and C_2. Signals were measured in the same cell after different dark adaptation time t_{DA} (1 min to 9 min) following a strong light-adapting illumination. At the same t_{DA} the relative size of the two components is different for voltage and current signals.



comes larger than C_1 at different dark adaptation times t_{DA} for current and voltage response: In a relatively light-adapted state (short t_{DA}) a certain light stimulus evoked a response the first component of which is greater than the second one; in a more dark-adapted state (longer t_{DA}, same light stimulus) the second component becomes greater than the first (see Fig. 9). The two components are diminished to different degrees by light adaptation and they differ in their recovery with dark adaptation. Since the transition from the first to the second phase of dark adaptation occurs when the second component becomes larger than the first, we assume that the two phases of sensitivity recovery during dark adaptation reflect the different time course of the recovery of the two components with dark adaptation. A further support for this assumption is a corresponding discontinuity in the change of t_{max} (time-to-peak) and t_{lat} (latency) for the voltage responses of criterion amplitude in dependence of dark adaptation time at the time t_{DA} when the second phase of dark adaptation

Fig. 9. The components C_1 and C_2 of the receptor potentials at the same light intensity (I/I_0 1/25, filter 5), but at different times t_{DA} after light adapting stimulus, showing the variation of the two components C_1 and C_2 with t_{DA}. (Cl 1A)

begins (Fig. 10) *i.e.*, when the second component of the response becomes larger than the first component. All these results indicate that the first phase of dark adaptation is determined by the recovery of the component C_1 and the second phase by the recovery of the component C_2 of the light response.

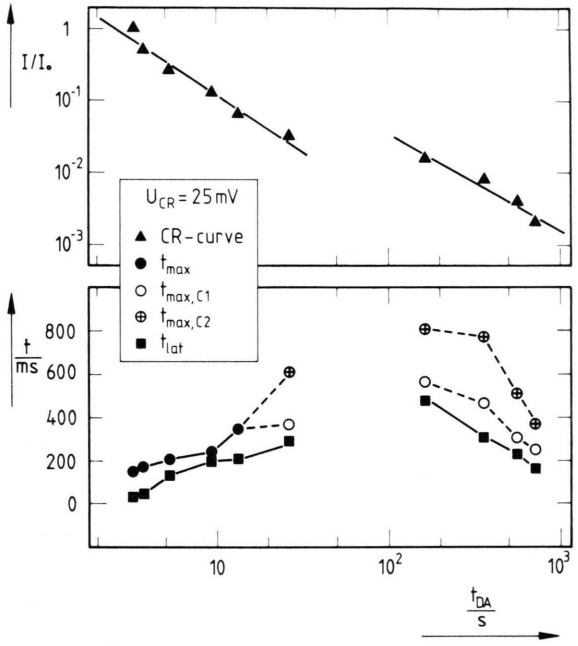


Fig. 10. Time course of dark adaptation.
Upper curve: reciprocal sensitivity for criterion response in PS (10 mmol/l Ca^{2+}); double logarithmic plot of normalized stimulus light intensity I/I_o to evoke voltage responses of criterion amplitude U_{CR} 25 mV versus dark adaptation time t_{DA}.
Lower curves: t_{lat} and t_{max} for each response of (about) criterion amplitude plotted versus log t_{DA}; t_{max} values were determined separately for C_1 and C_2. (Cl 1 A)

The dependence of t_{lat} and t_{max} on the time t_{DA} of dark adaptation is complex: For constant stimulus curves t_{lat} decreases more or less monotonically with t_{DA} (Fig. 11). However, for voltage responses of criterion height (U_{CR}) t_{max} and t_{lat} were relatively short, when U_{CR} was evoked by a strong light flash after a short dark adaptation time (t_{lat} was about 30–80 ms and t_{max} about 100–150 ms, Figs. 10 and 12). With longer dark adaptation times and therefore lower light intensities, t_{max} and t_{lat} increases up to a maximum (t_{lat} > 300 ms and t_{max} between 450 and 550 ms). But when the light intensity evoking U_{CR} is

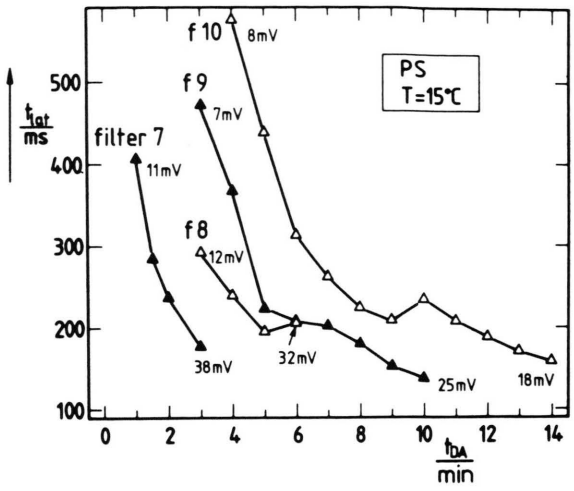


Fig. 11. Shortening of the latent period t_{lat} with dark adaptation time t_{DA} for four constant stimulus intensities (filter 7, 8, 9, 10). The peak amplitude h_{max} (first and last values listed) increases with longer dark adaptation times. (Cl 5 A)

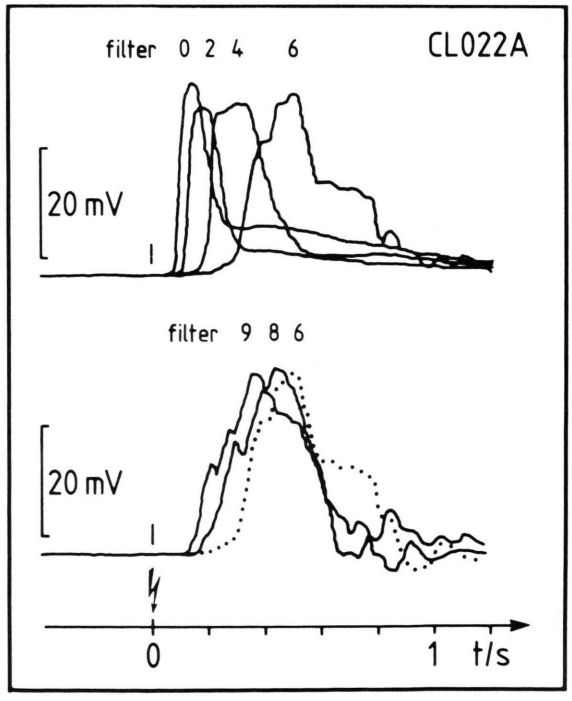


Fig. 12. Receptor potentials of approximately criterion height, evoked by test flashes of different light intensities and after different dark adaptation times. With increasing t_{DA} t_{lat} and t_{max} initially increase and later decrease. The response evoked by a filter 6 flash is drawn in both families of responses for comparison. (Cl 22 A)

Introduction and [3]). We conjecture that the intracellular Ca^{2+}-concentration controls the degree of the amplification involved in the visual transduction process via a yet unknown molecular mechanism.

The second, not calcium controlled adaptation process is observed when the intracellular Ca^{2+}-level is low and thus the sensitivity of the photoreceptor cell (already) relatively high. It has so far only been reported by our group [1, 5, 6]. It is demonstrated by three independent observations:

a) As described in this paper, the second phase of dark adaptation is not changed when the extracellular Ca^{2+}-concentration is varied.

b) This second phase of dark adaptation is not correlated to changes in intracellular Ca^{2+}-concentration measured by Arsenazo ([7], see above).

c) The directly observed diminution of bump size resulting from *weak* light adaptation shows no significant dependence on extracellular calcium concentration which was varied between 1 nmol/l and 100 mmol/l [9]. Even when the external calcium concentration was lowered as much as to 1 nmol/l, substantial light adaptation characterized by bump diminution is still observed; under these conditions no light-induced increase in intracellular Ca^{2+} can be detected by Arsenazo.

The mechanism of this not (primarily) calcium-controlled adaptation process is not known; we suspect that cyclic AMP might be the controlling agent.

Our results indicate that the differing recoveries of the two components of the light response are responsible for the two different phases in dark adaptation: during the first phase the light response is dominated by the component C_1, during the second by C_2. These two components have been studied by Maaz *et al.* [17] in the membrane voltage response and in the membrane current response to light. The reversal potential of the two components does not differ significantly. This indicates that both components are caused by a light induced conductance increase (ion channels) of the same ion specificity.

In recent studies [24, 25] it is demonstrated that the two components of the light response may be caused by the interaction of the summated bumps evoked by the light stimulus and an attenuation function a(t), resulting in a weighted bump sum. The time course of this attenuation function resembles, and may be identical with, that of the light induced increase in intracellular calcium concentration; this mechanism might act as an automatic gain control. This assumption provides an explanation for the calcium dependence of the first phase of dark adaptation which might be caused by variation of the degree of amplification depending on the intracellular calcium concentration present already at the time of the test flash. It also provides an explanation for the increase in latency and time-to-peak during dark adaptation [24]. It does not, however, provide an explanation for the increase in sensitivity during the second phase of dark adaptation and for the accompanying decrease of latency and time-to-peak. These effects will be studied further.

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reduced to about $10^{-2}-10^{-3}$ and correspondingly the dark adaptation time needed is relatively long (> 2 min), t_{max} and t_{lat} become shorter again (t_{lat} about 200 ms and t_{max} about 300–400 ms). The time-to-peak measured for responses of criterion height increases for both components C_1 and C_2 with increasing t_{DA} to a maximum and decreases with even longer dark adaptation times (see Fig. 10). This is also illustrated in Fig. 12, showing receptor potentials of approximately the same (criterion) amplitude after different dark adaptation times. Both t_{lat} and t_{max} (of C_1 and C_2) first increase with increasing t_{DA} and later decrease.

Discussion

The experiments described above show that during dark adaptation the sensitivity of the ventral photoreceptor recovers in two phases. The first phase is rather fast (20–30 s); the second, slower phase of sensitivity recovery is completed after about 15–20 min. These two phases of dark adaptation could also be shown in constant stimulus curves for the recovery of the amplitude of the receptor potential (see above, and [7, 15]). In addition we show here that only the first phase of dark adaptation is significantly influenced by changes in extracellular Ca^{2+}-concentration. During this first phase the sensitivity is raised and recovers somewhat faster when the external Ca^{2+}-concentration is lowered to 250 μmol/l. Raising the external Ca^{2+}-concentration to 40 mmol/l has the opposite effects. In contrast to this the second phase of dark adaptation is not (– or at least much less –) Ca^{2+}-dependent.

In response to the light-adapting stimulation the intracellular Ca^{2+}-concentration is raised transiently (see introduction). This rise in Ca^{2+}-concentration is smaller when the extracellular Ca^{2+}-concentration is lowered [18, 19] under experimental conditions similar to those used in our experiments discussed here.

Stieve, Bruns and Gaube [15] showed that the sensitivity of the dark adapted *Limulus* photoreceptor cell is not significantly changed, when the external calcium concentration was varied between 40 μmol/l und 100 mmol/l, whereas the sensitivity shift due to a light adapting illumination is reduced when the external calcium concentration is lowered, and augmented, when the calcium concentration is increased. These data indicate that the light-induced calcium increase in our experiments is the larger the higher the external calcium concentration is. Our results are in contrast to some of the results of Levy and Fein [20] who report that in their experiments lowering the $[Ca^{2+}]_{ex}$ leads to a decrease in the level of the $[Ca^{2+}]_i$ which is accompanied by a variable transient calcium-increase followed by a decrease in sensitivity. However, the light-induced increase in $[Ca^{2+}]_i$ was either equal or smaller when the $[Ca^{2+}]_{ex}$ was lowered. These authors suggest that under certain conditions $[Ca^{2+}]_i$ and sensitivity are uncoupled.

In the experiments discussed here, the desensitization due to the conditioning, light-adapting stimulation is smaller in low external calcium concentration and larger in high calcium concentration of the superfusate. This is probably the main reason for the difference in the dark adaptation during the first phase. The reduction in intracellular calcium concentration during dark adaptation is partially due to calcium sequestering in intracellular stores, but finally completed by non-electroneutral calcium transport out of the cell, probably due to an electrogenic sodium/calcium exchange mechanism [2, 19, 22, 23]. So we can assume that the final state of dark adaptation achieved in our experiments did not depend much on the external calcium concentration, whereas the degree of light adaptation was stronger in high, and weaker in low external calcium concentration.

Changes in intracellular Ca^{2+}-concentration can be measured by the calcium indicator Arsenazo injected into the photoreceptor cell [7, 18, 19, 21]. Nagy and Stieve [7] reported simultaneous measurements of the receptor potentials and the Arsenazo signals evoked by constant test stimuli during dark adaptation in the *Limulus* photoreceptor. Their measurements showed that during dark adaptation the intracellular Ca^{2+}-concentration – which has been elevated by light adaptation – returns monotonically to the low level of the dark adapted cell. The size of the receptor potential, however, recovers in two phases of dark adaptation. Only the first phase is correlated with the decrease in intracellular Ca^{2+}-concentration. The second phase of dark adaptation appears when the intracellular Ca^{2+} level has already returned to low values. These two phases of recovery seem to correspond to the two phases of dark adaptation characterized by measurements of sensitivity reported here.

The phenomenon of Ca-dependent light-dark adaptation has been reported by several authors (see

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