Light and Dark Adaptation of Crayfish Visual Cells Depending on Extracellular Calcium Concentration

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Lowering of extracellular Ca^{2+}-concentration from 14×10^{-3} M to ca. 10^{-6} M (using 1 mM ethyleneglycol bis (β-aminoethylether)-N,N′-tetraacetic acid (EGTA)) results in weaker light adaptation by a 2 s light exposure and faster dark adaptation (half time 45 ± 1%) of crayfish visual cells.

Introduction

Calcium ions are of specific significance for the light response of arthropode visual cells: They play an important role in the process generating the light induced conductivity change of the photoreceptor cell membrane which in turn causes the receptor potential (Stieve [1]; Weeks and Duncan [3]; Stieve [4]; Stieve [2]). Moreover the intracellular concentration of ionized calcium is probably an important factor controlling the sensitive change in the process of light and dark adaptation (Lisman and Brown [5, 6]; Brown and Blinks [7]; Fein and Lisman [8]; Hanani and Hillman [9, 10]). In this context we studied the influence of lowering the external Ca^{2+} concentration on the process of light and dark adaptation of crayfish visual cells.

Methods

Isolated retinas from compound eyes of the crayfish *Astacus leptodactylus* were superfused by streaming saline and the receptor potential was measured by means of extracellular electrodes according to a method described elsewhere (Stieve and Wirth [11]). After dissection the retina was kept in the dark in physiological saline (van Harreveld solution) which had the following composition: Na^+ 207.3, K^+ 5, Ca^{2+} 14, Mg^{2+} 3, Cl^- 244, HCO_3^- 2.3 mM. The retina was stimulated in regular intervals by constant white light flashes of 10 ms duration until the response reached a constant height. The intensity of the light stimuli was 750 lx, which corresponds to ca. 1.3×10^{15} photons·cm^{-2}·s^{-1}. After this pre-period, which normally lasted about 1 h, the retina was light adapted by a strong white light flash (30,000 lx resp. 5.1×10^{16} photons cm^{-2}·s^{-1}) of 2 s duration. The following dark adaptation was pursued for 60 min by checking the sensitivity of the retina using the same 10 ms light flashes already applied prior to the light adaptation.

In the second section of the experiment light and dark adaptation were repeated when the same retina was superfused with a saline of lowered calcium concentration. As compared to standard van Harreveld solution all ions including magnesium were kept constant except for calcium chloride which was omitted and replaced by sucrose; additionally 1 mM EGTA [ethyleneglycol bis (β-aminoethylether)-N,N′ tetraacetic acid] was added to the test solution. According to Portzehl *et al.* [12] the calcium content under these conditions is less than 10^{-6} M.

In a third section of the experiment the light and dark adaptation procedure was repeated with the retina again in normal physiological saline.

All experiments were carried out at approx. 15 °C.

From the recorded receptor potential various parameters were measured (see Table I). For comparison the values of the parameters are expressed in per cent of the value obtained from a reference potential in each experimental section, which was recorded immediately before application of the light-adapting 2 s flash (Fig. 1).

Results

All the 10 experiments carried out showed qualitatively the same results. Only three experiments were performed in such a way that an exact quantitative comparison and averaging was possible. The results are shown in Figs 1 and 2 and Table I.

Due to the reduction of external Ca^{2+} concentration the receptor potential of the incomplete dark adapted retina changes (Table I) in agreement with

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Table I. Values (mean and S.E.) of measured parameters of receptor potentials during light/dark adaptation of *Astacus* retina in normal (pS) and low calcium (EGTA) environment.

h_{max}, maximum amplitude of receptor potential (ReP);
t_{lat}, latency — time from light flash onset until the first visible increase of the receptor potential;
t_{max}, time-to-peak — time from light flash onset until the maxium is reached;
t_2, repolarization time — time in which the receptor potential decreases from h_{max} to $h_{max}/2$.
Reference value: last ReP before light adaptation (LA). Level value: level of h_{max} of ReP, reached approx. 30—40 min after LA.
$t_{1/2}$, time from LA until h_{max} reaches half height of level value (the values in this column are determined by interpolation);
LA, light adaptation 2 (or 1) s white light (30,000 lx);
DA, dark adaptation tested with short light flashes (750 lx) of 10 ms duration;
$n=3$ experiments.

	Mean reference value		First value after LA		$t_{1/2}$ of DA course		Level value of DA	
	p.S.	EGTA	p.S.	EGTA	p.S.	EGTA	p.S.	EGTA
h_{max}	0.54 ± 0.03 mV	0.55 ± 0.04 mV	0.8 ± 0.8%	11 ± 1.8%	9 ± 2.8 min	4 ± 1.3 min	87 ± 5.0%	88 ± 6.2%

	Mean reference value		First value after LA		Value at $t_{1/2}$ of DA		Value at plateau of DA	
	p.S.	EGTA	p.S.	EGTA	p.S.	EGTA	p.S.	EGTA
t_{lat}	4 ± 1.5 ms	4 ± 1.2 ms	(194%)*	218 ± 66%	164 ± 9.1%	188 ± 23%	122 ± 12%	165 ± 23%
t_{max}	17 ± 4.7 ms	21 ± 4.5 ms	(66%)*	104 ± 40%	94 ± 11%	119 ± 44%	96 ± 6.5%	125 ± 15%
t_2	394 ± 58 ms	768 ± 449 ms	(219%)*	170 ± 101%	32 ± 6.4%	52 ± 6.4%	62 ± 2.1%	103 ± 19%

* Only 1 experiment shows a visible response to the first light flash after 2 s LA.
The values (except $t_{1/2}$) are given in per cent of the respective reference value.

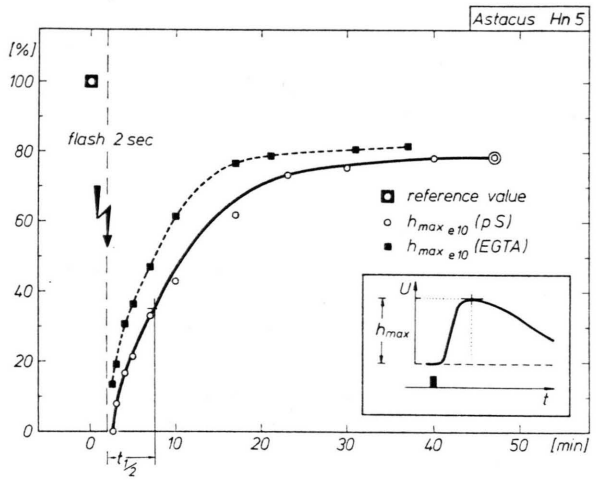

Fig. 1. Time course of light/dark adaptation of an *Astacus* retina in normal and low calcium environment (one experiment). Level value (in pS) marked with a double ring. Reference value marked with a square. pS, physiological saline; EGTA, low Ca^{2+} saline (see text).

our results described earlier (Stieve and Wirth [11]). The amplitude h_{max} of the receptor potential and the latency t_{lat} show only negligible differences; the time-to-peak t_{max} is somewhat prolonged, but the most prominent difference is the retarded repolarization: the decrease-time t_2 is almost doubled.

In contrast to visual cells of the *Limulus* lateral eye, which become inexcitable in solutions of such low external calcium concentration ($<10^{-6}$ M) (Stieve [2]), the crayfish retina still responds under such conditions; since calcium can partially be replaced in action by the available magnesium.

The reduction of the height h_{max} of the light response due to the 2 s light adaptation caused by the test stimulus is smaller in low calcium environment than at normal Ca^{2+} concentration (Table I).

During the course of the following dark adaptation the response height h_{max} increases with time and reaches an almost constant level in about 30 min. This "level value" (marked with a double ring in Fig. 1) of the response height was about the same for conditions of normal and low calcium (Table I). This "level value" after 30 min dark adaptation, however, has not yet reached the same height as the reference value before light adaptation (Table I and Fig. 1).

The process of dark adaptation is faster in low calcium solution than in normal solution. The half-time $t_{1/2}$ for reaching the level value (Fig. 1), which is 9 ± 2.8 min in physiological saline is decreased to 45 ± 1% under low calcium conditions. The difference is sinificant ($p=0.0006$).

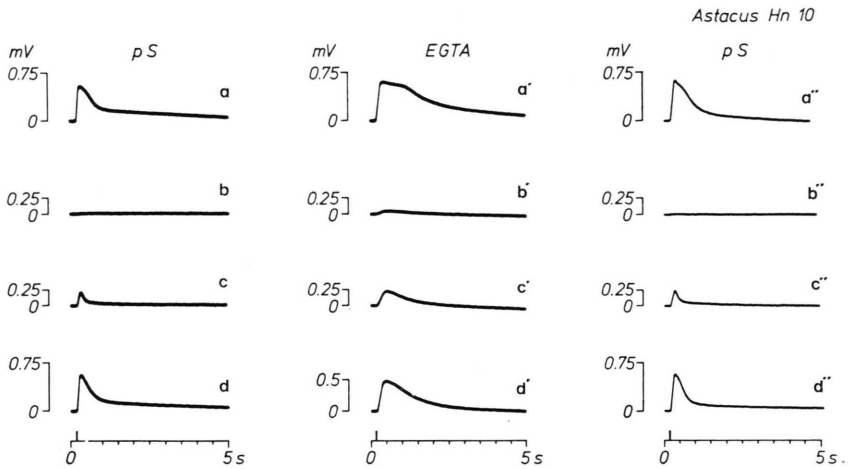

Fig. 2. Receptor potentials of an *Astacus* retina during light and dark adaptation in normal, in low and again in normal calcium environment. First column: physiological saline. Second column: EGTA solution. Third column: physiological saline.
a a′ a″ reference value; last receptor potential before LA.
b b′ b″ first receptor potential 30 s after the 1 s light adapting flash.
c c′ c″ receptor potential at the time $t_{1/2}$ (h_{max} reaches half the height of the level value).
d d′ d″ receptor potential with h_{max} at level value.

In Table I the measured parameters of the receptor potential are compared at three specified points during the course of adaptation: 1. first measurement 30 s (except in one case 60 s) after the light-adapting illumination; 2. at the time when the response height h_{max} is reduced to half the level value ($t_{1/2}$ of DA course; here the values were determined by interpolation); 3. at the level value about 30 min after light adaptation.

As can be seen from the values at $t_{1/2}$ of the DA course, light adaptation – besides decreasing the response height h_{max} – causes an increase of the latency t_{lat}, shortens the decrease-time t_2, but does not lead to a significant change of the time-to-peak t_{max}. Differences in the relative adaptative changes of these parameters in low calcium medium as compared to normal Ca^{2+} concentration are not significant.

Discussion

According to a hypothesis of Lisman and Brown [6] and Brown and Blinks [7] the rise in intracellular concentration of ionized calcium $[Ca^{2+}]_{in}$ mediates the sensitivity decrease during light adaptation of *Limulus* ventral photoreceptor cells. Brown and Blinks [7] observed a light induced increase of $[Ca^{2+}]_{in}$ in *Limulus* and barnacle photoreceptor cells. In *Limulus* ventral photoreceptors this increase does not depend on the external Ca^{2+} concentration $[Ca^{2+}]_{ex}$ whereas in the lateral photoreceptors of the barnacle it is reduced when $[Ca^{2+}]_{ex}$ is lowered. Brown and Blinks [7] suggested that the light-induced increase of $[Ca^{2+}]_{in}$ leads to reversible binding of calcium to intracellular sites of high affinity which in turn could cause a reduction of the light induced increase of Na^+ conductivity of the membrane. Fein and Lisman [8] injected Ca^{2+} locally into ventral photoreceptors of *Limulus* and obtained results similar to local light adaptation. Their findings strongly support the hypothesis described. Low $[Ca^{2+}]_{ex}$ was found to reduce light adaptation considerably and to increase the rate of dark adaptation in barnacle photoreceptors (Hanani and Hillman [9, 10]). They suggested that an increase in $[Ca^{2+}]_{in}$ as a result of light induced influx of Ca^{2+} causes light adaptation by blocking sodium channels.

The function of the arthropod visual cell membrane can be described in the simplest way by assuming the existence of permanently open "dark channels" which let preferably K^+ ions pass and "light channels" which are opened only transiently in consequence of stimulation by light and which let preferably Na^+ ions pass (Stieve [2]).

According to our concept (Stieve [2, 4]) the control of the conductivity of these light channels involves a calcium/sodium binding competition at the cell membrane. Up to now there is no decisive proof whether the binding sites are located at the outer or inner membrane surface or whether they are located in the interior of the cell membrane.

In this context one can follow an analogous argumentation outlined by Hildebrand and Dryl [13] for the location of Ca^{2+} binding sites at the cell membrane of *Paramecium*: It is a probable assumption that the Ca^{2+} permeability of the arthropod visual cell membrane is low in the dark. Under this presumption the results of intracellular Ca^{2+} injection obtained by Fein and Lisman [8] suggest that Ca^{2+} binding sites controlling adaptation are located at

the inner surface of the cell membrane. Our results (Stieve [2, 4]) from experiments reducing the external Ca^{2+} concentration in the dark suggest Ca^{2+} binding sites, controlling the membrane conductivity located at the outer surface of the cell membrane; their sites can be occupied competetively by Na^+. Binding of Ca^{2+} at the outer or at the inner surface of the photoreceptor cell membrane thus may have similar effect on the light induced change of membrane permeability. However the sensitivity change during light and dark adaptation should be controlled *via* the calcium binding sites at the intracellular membrane surface. Under physiological normal conditions the relative changes in Ca^{2+} concentration due to the light response and during light and dark adaptation are probably much greater intracellularly than extracellularly.

The simplest explanation of the weaker light adaptation at low external Ca^{2+} concentration as shown by our experiments is that at low $[Ca^{2+}]_{ex}$ the light induced increase in $[Ca^{2+}]_{in}$ is smaller than under physiological normal conditions, due to reduced Ca^{2+} influx. In the course of dark adaptation the sensitivity is restored by a decrease of $[Ca^{2+}]_{in}$ which is brought about by processes like calcium outward transport or sequestering of calcium in mitochondria. The observed faster dark adaptation at low $[Ca^{2+}]_{ex}$ could be explained if the rate of decrease in $[Ca^{2+}]_{in}$ (which was increased by light adaptation) is determined by the capacity of the processes lowering $[Ca^{2+}]_{in}$, *e. g.* by the capacity of active calcium transport. Such a system removes smaller increases in $[Ca^{2+}]_{in}$ faster than greater increases.

According to Lisman and Brown [5] $[Ca^{2+}]_{in}$ can increase during the light response for two different reasons:

1. light induced Ca^{2+} influx;
2. light induced Na^+ influx, which causes a release of Ca^{2+} from intracellular sources.

A light induced Ca^{2+} influx could be demonstrated in the photoreceptor of the barnacle, a crustacean, but not in the ventral photoreceptor of *Limulus* (Brown and Blinks [7]).

Probably the crayfish photoreceptor membrane behaves more like that of the barnacle rather than like that of *Limulus*.

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