Ability to Light-Induced Conductance Change of Arthropod Visual Cell Membrane, Indirectly Depending on Membrane Potential, during Depolarization by External Potassium or Ouabain *

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(Z. Naturforsch. **32 c**, 855–869 [1977]; received July 12, 1977)

Astacus and *Limuluis* Photoreceptors, Light Response, Membrane Conductance, Ouabain, Potassium Depolarization

Light responses (ReP) and pre-stimulus membrane potential (PMP) and conductance of photoreceptors of *Astacus leptodactylus* and *Limulus polyphemus* (lateral eye) were recorded and changes were observed when the photoreceptor was depolarized by the action of external ouabain or high potassium concentration application.

1 mM/l ouabain application causes a transient increase of PMP and ReP in *Limulus*, followed by a decrease which is faster for the ReP (half time 34 min) than for the PMP (half time 80 min). Irreversible loss of excitability occurs when the PMP is still ca. 40% of the reference value.

In both preparations high external potassium concentration leads to total depolarization (beyond zero line to +10 – +20 mV) of the PMP and after a time lag of 10 min also to a loss of excitability (intracellular recording). In extracellular recordings (*Astacus*) the excitability remains at a low level of 15%. The effects are reversible and are similar whether no or 10% external sodium is present. In all experiments the light-induced changes of membrane conductance are about parallel to those of the light response.

The fact that the ability of the photosensoric membrane to undergo light-induced conductance changes is membrane potential-dependent is discussed, leading to the explanation that dipolar membrane constituents such as channel forming molecules (probably not rhodopsin) have to be ordered by the membrane potential to keep the membrane functional for the photosensoric action.

Introduction

In the nerve membrane the sodium conductance changes during excitation occur only if the resting membrane potential is sufficiently high. At lower membrane potentials following depolarization by raising the external potassium concentration (Cole and Curtis [2]) the sodium permeability system is inactivated. Hodgkin and Huxley [3] found that depolarization by 20 mV inactivates more than 50% of the sodium conductance system of the squid nerve membrane.

As opposed to the axon membrane the visual cell membrane is generally not electrically excitable – except for the fact that a small component of the light response can be elicited by an electrical depolarizing step (Purple and Dodge [4], Millecchia and Mauro [5]).

In the arthropod photoreceptor a light-induced change of the membrane conductance causes the voltage signal at the cell membrane (Fuortes [6], Stieve *et al.* [7]). In order to interpret earlier experiments dealing with ouabain poisoning of visual cells (Stieve [8, 1]) we wanted to study whether the ability to permeability change of the visual cell membrane during the light response depends on the membrane potential. To this end photoreceptors of *Astacus leptodactylus* and *Limulus polyphemus* (lateral eye) were exposed to the depolarizing effect of ouabain or high external potassium concentration. In part of the experiments light responses were recorded simultaneously by intra- and extracellular electrodes from the same preparation to get information on differences of these two recording methods.

Methods

Slices of lateral eyes of the horseshoe crab *Limulus polyphemus* and of isolated retinas of the crayfish *Astacus leptodactylus* were superfused in the experiments by a stream of test saline at a flow rate of 0.5 ml/min. The half time of the change of saline (until ca. 50% of the saline was exchanged at the preparation) was 10 min for *Limulus* and less for *Astacus*. (For a description of the preparation technique and other details see Stieve *et al.* [9].)

The ionic composition of the test salines is given in Table I. The crayfish retinas were kept in van

* Some of the results of this paper are already briefly described by Stieve [1].

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Table I. Ionic composition [mM/l] of salines used in the experiments.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	Ouabain
Limulus								
L_1 (physiological saline)	488.3	10.0	10.0	55.0	566.0	30.0	2.3	—
L_2 (ouabain added)	488.3	10.0	10.0	55.0	566.0	30.0	2.3	1.0
L_3 (K^+ added)	—	498.3	10.0	55.0	566.0	30.0	2.3	—
Astacus								
A_1 (physiological saline)	207.3	5.0	14.0	3.0	244.0	—	2.3	—
A_2 (K^+ added)	—	212.3	14.0	3.0	244.0	—	2.3	—
A_3 (K^+ added, Na^+ decreased to 10%)	20.7	191.6	14.0	3.0	244.0	—	2.3	—

Harreveld's [10] solution, and the *Limuli* in artificial seawater (Table I), both at 15 °C. When the potassium concentration was increased a corresponding amount of sodium was omitted to keep the osmotic pressure constant. The pH of all salines was 7.5 to 8.0.

For intracellular measurement single visual cells were penetrated by glass microelectrodes filled with 0.5 M KCl solution, and the light response was recorded against an extracellular indifferent silver chloride electrode. For extracellular measurement two chlorided silver wires were in contact with the distal and proximal sides of the retina slice.

The electronic recording system allowed DC compensation, current injection through the microelectrode, and compensated current pulses through the measuring circuit in order to measure the input impedance (of cell membrane, cell and electrode in series). The data were FM recorded on magnetic analogue tape (Ampex FR 1300) and photographed for evaluation by hand. They were parallelly recorded by a paper recorder (Helcoscriptor HE 16). The time resolution of the system was 1 ms, the measuring accuracy was 1 mV (intracellular recording) and 0.01 mV (extracellular recording).

In the experiments labelled JA a xenon high pressure lamp (Osram XBO 900 W) with a maximal intensity of ca. 20000 lx (3.2×10^{16} photons·cm^{-2}·sec^{-1}), and in the experiments labelled JB a mercury super pressure lamp (Philips CS 100 W/2) with a maximal intensity of ca. 5500 lx (9×10^{15} photons·cm^2·sec^{-1}) was used. Stimuli of maximal intensity and two durations were applied: short stimuli ($\tau = 10$ ms for *Astacus*, $\tau = 33$ or 50 ms for *Limulus*) and long stimuli ($\tau = 1000$ ms).

Procedure

All experiments lasted at least three hours and were carried out at 15 °C in the dark. During a pre-period of 60 min constant stimuli every 10 or 5 min were applied. In the main period (60 to 120 min) the preparation was exposed to the test saline, and in an after-period of at least 60 min the original physiological saline was applied again.

Evaluation

In the intracellular recordings the membrane potential of the visual cell before the light stimulus could be measured (pre-stimulus membrane potential PMP; in the present experiments identical with the dark potential DaP; in the figure both abbreviations are used). The light responses (receptor potential, ReP) were evaluated concerning their size and shape by measuring the following parameters.

a) For short stimuli (τ shorter than latent period):

h_{max} [mV], maximal amplitude of transient;

t_{lat} [ms], latent period, from light flash onset until intersection of tangent line in point of steepest increase of rising phase with extrapolated level of base line;

t_{max} [ms], time – to – peak (from light flash onset until response maximum);

t_2 [ms], time of half decrease (from h_{max} to $h_{max/2}$);

b) for long stimuli (t = 1000 ms), additionally to h_{max} and t_{max}:

h_e [mV], plateau-value (at the end of the stimulus);

h_a [mV], response amplitude 500 ms after the end of the stimulus;

h_{max}/h_e, shape quotient.

The changes of the ReP were expressed in per cent of the reference values of the last ReP of the pre-period.

Additionally impedance measurements (of the input resistance, *i. e.* of the sum of the resistances of cell membrane, cell and electrode in series) were carried out using current pulses (0.5 – 1.5 nA) through the intracellular electrode (20 ms duration and 10 Hz frequency for short stimuli, 50 ms and 5 Hz for long stimuli). Measurements of the input impedance in the dark (R_d) and its changes at various times of the ReP (ΔR_m at the time of the maximal amplitude, etc.) are listed in some tables and figures. The values were normalized similarly as described for the receptor potential parameters.

Results

1. Ouabain

In earlier experiments we could show by extracellular recording (Stieve *et al.*[7]) that under influence of ouabain the excitability of the visual cells of the *Astacus* retina disappears; this happens the faster, the stronger and more frequently the cells are stimulated by light. Intracellular recordings in *Limulus* retinular cells showed that the cells were inexcitable due to ouabain poisoning long before the dark potential had become zero (Stieve[8]). This is in agreement with the results of Smith *et al.*[11] and Brown and Lisman[12] (who were using strophanthidin).

1.1. *Limulus*

In 7 experiments (JA 1 – 5, JB 39, JB 45) receptor potential and dark potential were intracellularly measured under the influence of ouabain (1 mM/l added to the saline). In 2 experiments (JB 39, JB 45) dark resistance and light-induced resistance change were additionally measured (Table II, Figs 1 – 3).

Due to the action of ouabain (responses shown in Fig. 1) receptor potential and dark potential showed a transient increase in the first minutes (receptor potential in most cases, dark potential always) which was followed by a continuous gradual decrease (Fig. 2). The half-time for the decrease of the ReP was 34 min, for the PMP 80 min on the average (Table II). Washing out the poison did not lead to recovery of the cell, in contrast to earlier findings with unpenetrated cells of the *Astacus* retina (external electrodes; Stieve *et al.*[7]). In those cases in which the PMP had not already reached zero in the main period it decreased further in the after-period. It never recovered in the after-period. In Table II the parameters of the receptor potential measured when h_{max} had decreased to

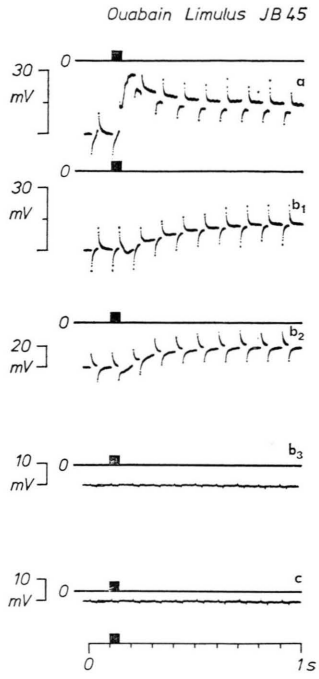

Fig. 1. Intracellularly recorded receptor potentials of a *Limulus* retinular cell and simultaneous measurement of the membrane resistance change ΔR (difference between the two recorded traces of each measurement, *i. e.*, height of the square-wave impulses). Light intensity ca. 5500 lx, stimulus duration $\tau = 50$ ms. a: in physiological saline; b_1: 40 min in test saline containing 1 mM/l ouabain; b_2: 70 min in ouabain saline; b_3: 115 min in ouabain saline; c: 55 min again in physiological saline. JB 45, *Limulus* retinular cell.

50%, 30% and 0% of the reference value are compared. Also the values after 60 min in ouabain saline, at the end of the main period and during the after-period are listed. In Table II and Fig. 3 one can see that the ReP is already zero when the PMP has decreased to ca. 40% of its reference value. With decreasing h_{max} the time parameters t_{lat} and t_{max} (short stimuli) increase, whereas the decrease-time t_2 is shortened. For long stimuli h_e decreases slightly less than h_{max}, the shape-quotient h_{max}/h_e decreases, and t_{max} increases too.

The dark resistance does not change significantly under the action of ouabain. The light-induced resistance change ΔR_m decreases under the action of ouabain to a value no longer measurable even before the response has disappeared. No recovery of the light-induced conductance change could be found. Since the ReP and ΔR_m disappear at approx. the same time (while the PMP is still at a value of 40%) it is reasonable to assume that the ReP cannot

Table II. Dependence of intracellularly recorded pre-stimulus membrane potential (PMP_{corr}, for details of correction of PMP see Stieve *et al.*[9]), receptor potential (both JA 1—5) and membrane resistance (JB 39, JB 45) of *Limulus* retinular cells on 1 mM/l ouabain added to the physiological saline. Stimulus intensity ca. 20,000 lx (JA 1—5) and ca. 5500 lx (JB 39, JB 45).

1) $\tau = 33.3$ ms

	PMP_{corr} ($n=7$)	h_{max} ($n=7$)	t_{lat} ($n=7$)	t_{max} ($n=7$)	t_2 ($n=7$)	R_d ($n=2$)	ΔR_m ($n=2$)
reference value in physiological saline	-32 ± 4.2 mV	25 ± 4.9 mV	43 ± 8.7 mV	$239 + 91$ ms	209 ± 23 ms	36 ± 12 MΩ	7.7 ± 0.64 MΩ
values in test saline at							
h_{max} 50%	$(-)84 \pm 9.6\%$	50%	$174 \pm 33\%$	$182 \pm 49\%$	$48 \pm 4.4\%$	$63 \pm 24\%$	$48 \pm 21\%$
h_{max} 30%	$(-)64 \pm 10\%$	30%	$210 \pm 31\%$	$188 \pm 40\%$	$49 \pm 3.3\%$	$99 \pm 6.1\%$	$24 \pm 9.2\%$
h_{max} 0%	$(-)39 \pm 10\%$	—	—	—	—	$94 \pm 3.3\%$	—
55 min in test saline	$(-)62 \pm 16\%$	$43 \pm 20\%$	$194 \pm 59\%$	$128 \pm 26\%$	50%	—	—
last value in test saline	$-38 \pm 8.1\%$	$(-)1 \pm 1.8\%$ ($n=5$)	—	—	—	—	—
55 min in physiological saline	$33 \pm 13\%$	—	—	—	—	$134 \pm 115\%$	—

2) $\tau = 1000$ ms

	PMP_{corr} ($n=7$)	h_{max} ($n=7$)	t_{max} ($n=7$)	h_e ($n=7$)	h_a ($n=7$)	h_{max}/h_e ($n=7$)	R_d ($n=2$)	ΔR_m ($n=2$)	ΔR_e ($n=2$)
reference value in physiological saline	-29 ± 3.2 mV	27 ± 5.6 mV	451 ± 142 ms	15 ± 1.8 mV	12 ± 2.2 mV	2.2 ± 0.76	37 ± 14 MΩ	7 ± 1.8 MΩ	5.5 ± 0.78 MΩ
60 min in test saline	$57 \pm 20\%$	$21 \pm 12\%$	$202 \pm 129\%$	$23 \pm 11\%$	$24 \pm 11\%$	$71 \pm 18\%$	—	—	—
last value in test saline	$38 \pm 11\%$	$5 \pm 17\%$	$221 \pm 184\%$	$15 \pm 12\%$	—	—	—	—	—
60 min in physiological saline	$27 \pm 2.2\%$	—	—	—	—	—	—	—	—

The half-time of the depolarization is $t_{1/2} = 34 \pm 4.3$ min for h_{max} and $t_{1/2} = 80 \pm 34$ min for the PMP. The signs in brackets indicate the polarity of the PMP.

Table III. Dependence of intracellularly recorded pre-stimulus membrane potential (PMP_{corr}), receptor potential and membrane resistance of *Limulus* retinular cells on 498 mM/l potassium added to the physiological saline. Stimulus intensity ca. 1000 lx. JB 46–50, $n=5$.

1) $\tau=50$ ms

	PMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	47 ± 4.3 mV	45 ± 2.0 mV	38 ± 7.4 ms	165 ± 8.4 ms	749 ± 209 ms	44 ± 11 MΩ	−10 ± 1.7 MΩ
values in test saline at							
h_{max} 50%	(−) 13 ± 21%	50%	215 ± 33%	131 ± 19%	116 ± 19%	77 ± 2.5%	36 ± 5.5%
h_{max} 30%	(+) 5 ± 23%	30%	279 ± 71%	147 ± 23%	98 ± 18%	68 ± 2.7%	20 ± 3.3%
h_{max} 0%	(+) 25 ± 19%	—	—	—	—	60 ± 2.2%	—
55 min in test saline	(+) 38 ± 21%	2 ± 1.4%	570 ± 431%	221 ± 133%	131 ± 119% ($n=2$)	55 ± 3.4%	—
last value in test saline	(+) 14 ± 16%	—	—	—	—	—	—
55 min in physiological saline	(−) 55 ± 28%	23 ± 14%	180 ± 100%	135 ± 51%	63% ($n=1$)	67 ± 4.6%	17 ± 11%

2) $\tau=1000$ ms

	PMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	−46.0 ± 0.30 mV	47 ± 2.0 mV	188 ± 11 ms	36 ± 1.6 mV	18 ± 2.8 mV	1.79 ± 0.11	44 ± 11 MΩ	10 ± 1.6 MΩ	8.4 ± 1.4 MΩ
60 min in test saline	(+) 29 ± 18%	4 ± 1.8%	224 ± 91%	4 ± 1.6%	4 ± 2.4%	112 ± 28%	—	—	—
last value in test saline	(+) 14 ± 21%	5 ± 2.0%	301 ± 108%	5 ± 2.5%	5 ± 4.0%	—	—	—	—
60 min in physiological saline	(−) 10 ± 42%	29 ± 17%	347 ± 152%	42 ± 24%	53 ± 30%	67 ± 3.9%	—	—	—

The half-time of the depolarization is $t_{1/2}=15\pm1.0$ min for h_{max} and $t_{1/2}=13\pm1.3$ min for the PMP. The signs in brackets indicate the polarity of the PMP.

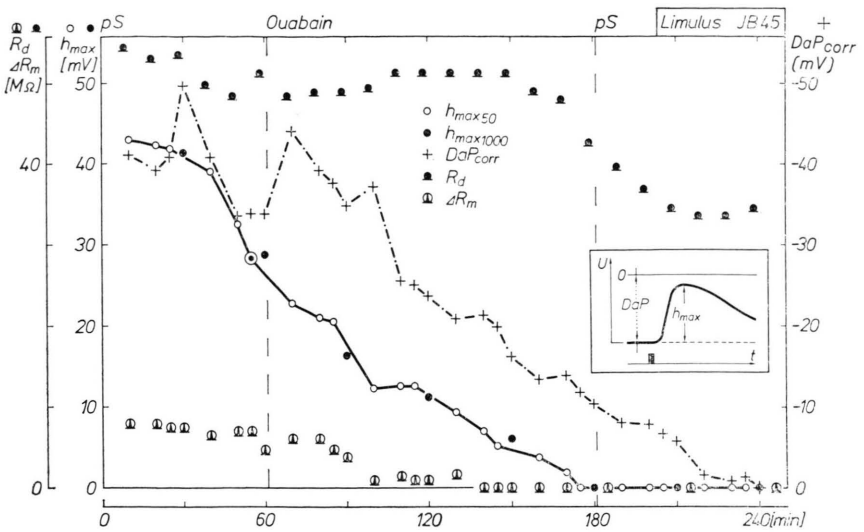

Fig. 2. Time course of the effect of ouabain poisoning of *Limulus* retinular cell. Intracellular measurement of h_{max50} [mV] (short stimuli), $h_{max1000}$ [mV] (long stimuli), DaP$_{corr}$ [mV], R_d (MΩ) *vs* time [min]. pS: perfusion with physiological saline; ouabain: perfusion with test saline containing 1 mM/l ouabain. For further details see Fig. 1. JB 45, *Limulus* retinular cell.

be produced when there is no more light-induced conductance change.

Earlier emperiments with the ventral photoreceptor of *Limulus* confirm the observation that the ReP disappears when the PMP is still existent (Stieve and Appelhans [13]).

1.2. *Astacus*

No intracellular experiments on the action of ouabain on the *Astacus* visual cell were made. In former experiments already published (Stieve *et al.* [7]) only extracellular electrodes were used. Those experiments are in good agreement with the experiments reported here on *Limulus*, except that under the action of ouabain, when the ReP is decreasing, both t_{lat} and t_{max} decrease in *Astacus* contrary to the intracellular measurements in *Limulus*. The most important difference, however, is that the loss of excitability after poisoning is reversible in the extracellular recording, where the ReP reappears within 30 min after washing out the ouabain, and starts to recover slowly.

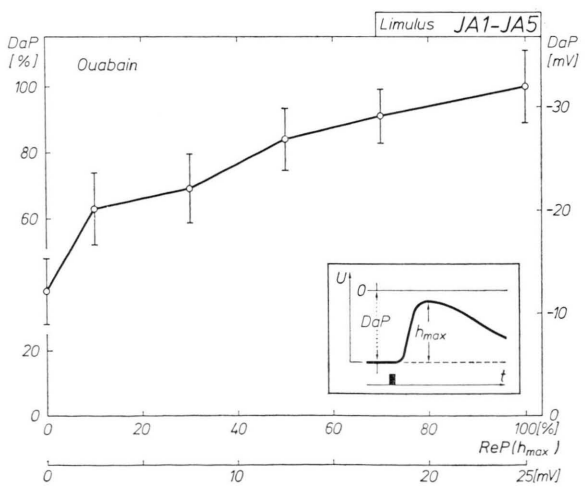

Fig. 3. Intracellular measurement of pre-stimulus membrane potential PMP=DaP [mV] *vs* maximal amplitude h_{max} [%] of reference value recorded in physiological saline of receptor potentials recorded in test saline containing 1 mM/l ouabain. Left scale: DaP in % of reference value. Scale below: h_{max} [mV]. Light intensity ca. 20,000 lx, stimulus duration τ=33 ms. The horizontal bars indicate the S.E. of the mean. JA 1–5, *Limulus* retinular cells.

2. *Depolarization by high extracellular potassium concentration*

Under the action of ouabain the visual cell membrane loses its ability to undergo light-induced conductance changes. The effect of ouabain on the dark potential, which is detectable only after a considerable time (half-time 80 min), could either be an indirect influence via decreasing ionic gradients, especially the sodium gradient, or a more direct action on the conductance change performing system. To answer this question we depolarized the visual cell by raising the extracellular potassium concentration. In earlier experiments we had shown that the extracellularly measured ReP of crustacean visual cells is not totally abolished by high external potassium concentration (Stieve [14], Stieve and Wirth [15]). Changing the extracellular potassium concentration should depolarize the membrane potential much faster than the action of ouabain.

In one set of experiments (*Astacus*) we additionally varied the sodium content (0% or 10% of normal concentration) of the external solution,

since the presence of extracellular sodium ions might be important for the conductance change performing system.

2.1. *Limulus*

In five experiments the influence of high external potassium on *Limulus* retinular cells was investigated by intracellular electrodes. In the test saline (L 3, Table I) all sodium was replaced by potassium while the content of the other ions was unchanged (Table III, Figs 4, 5).

Due to the change of the potassium gradient across the cell membrane the PMP decreases quickly and reverses polarity (indicated by the change from minus to plus values in Table III), reaching values as high as (+) 38% after 55 min in the test saline. The last value in the test saline is (+) 14%. The standard deviations of the PMP are very high, but the changes are still significant.

When the PMP is decreased to zero or reverses sign, the ReP and the light-induced resistance changes also slowly disappear, but a response of about 30% (h_{max}) of the reference value is still recorded when the PMP is already beyond the zero level (Fig. 5). Finally, after a time lag of more than 10 min after total depolarization of the membrane, ReP and light-induced resistance change disappear (Fig. 4). The half-times of the effects on PMP and ReP are practically the same (13 min for the PMP and 15 min for h_{max}).

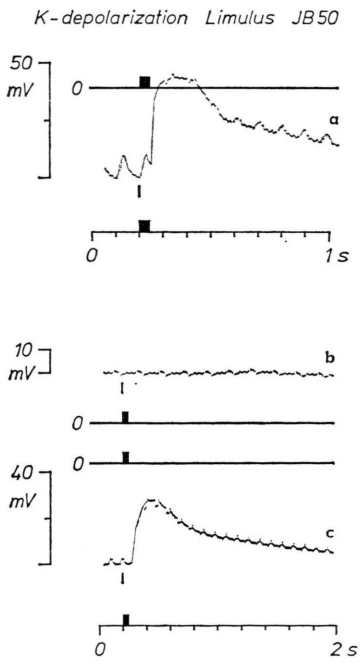

Fig. 4. Intracellularly recorded receptor potentials of a *Limulus* retinular cell and simultaneous measurement of the membrane resistance change ΔR. Light intensity ca. 1000 lx, stimulus duration $\tau = 50$ ms. a: in physiological saline; b: 55 min in test saline containing 498.3 mM/l potassium; c: 55 min again in physiological saline. JB 50, *Limulus* retinular cell.

The observed effects were reversible. After one hour in normal physiological saline the PMP had come back to (−) 50%, the ReP to about 25%. The restoration of the excitability (ReP and light-induced resistance change) follows the restoration of the PMP with almost no delay.

The time parameters (Table III) which had, in the test saline, either increased (t_{lat} 200%, t_{max} 150%) or not changed much (t_2) recover less in the after-period than the PMP or the ReP amplitudes.

Resistances

Under the influence of high external potassium the dark resistance R_d decreases to ca. 60% (Fig. 5). The light-induced resistance change at the time of the maximum of the ReP, ΔR_m, is still measurable when the PMP is already zero. It becomes immeasurable when h_{max} is zero. Both R_d and ΔR_m tend to recover in the after-period. As shown in Table III the decrease of ΔR_m corresponds fairly well with that of h_{max}. The changes seem to be correlated and have a common zero point. This means that there is no measurable light-induced conductance change when the ReP is reduced to zero.

2.2. *Astacus*

Potassium depolarization, no sodium

In 5 experiments (JB 52 – 56) electrical properties of *Astacus* visual cells were intracellularly measured in a test saline (Table I, A_2) of high external potassium content, without sodium. Table IV and Fig. 6 show the results.

The results are almost identical with those obtained from *Limulus*. The PMP decreases and changes sign in the test saline, it goes down to ca. (+) 25% (Table IV) with a half-time of 8 min, which is a little bit faster as compared to *Limulus*. The ReP decreases also faster (half-time 3.3 min). Also in the experiments with *Astacus* there is a time lag of more than 10 min after reversal of polarity of the membrane potential (PMP) until the cell becomes inexcitable. With decreasing h_{max} t_{lat} increases to ca. 140%, t_{max} decreases slightly, and t_2 decreases markedly.

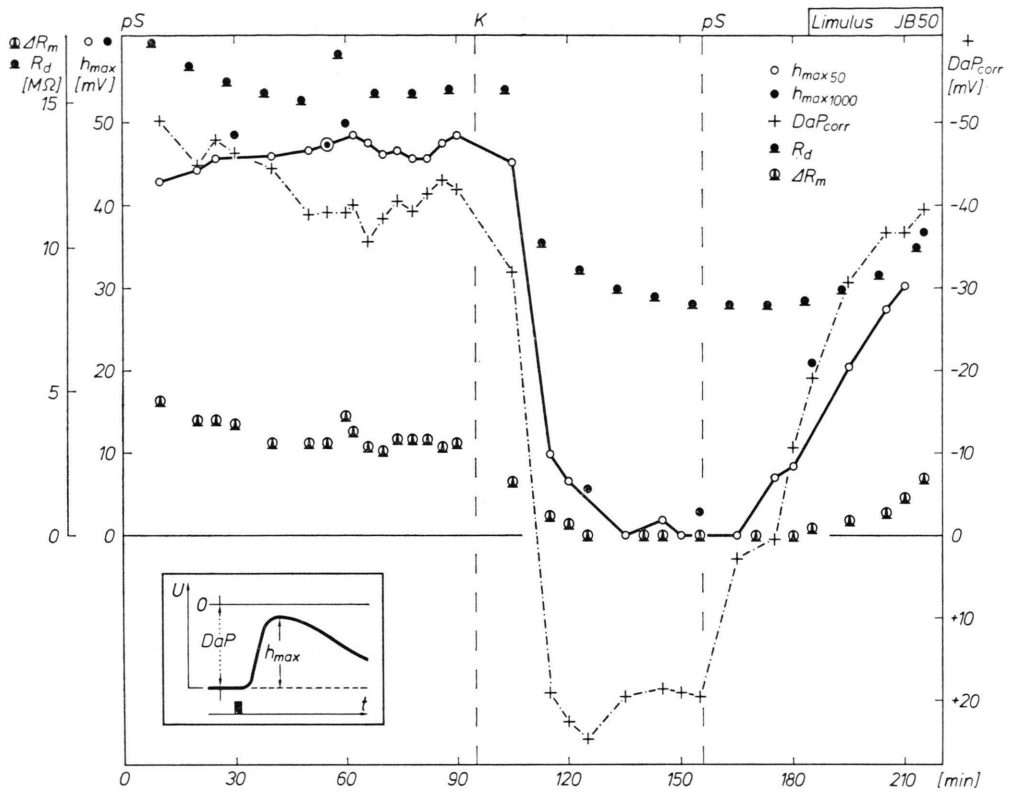

Fig. 5. Time course of the effect of potassium depolarization on *Limulus* retinular cell. Intracellular measurement of $h_{max_{50}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV], R_d [MΩ], and ΔR_m [MΩ] *vs* time [min]. pS: perfusion with physiological saline; K: perfusion with test saline containing 498.3 mM/l potassium, no sodium. For further details see Fig. 4. JB 50, *Limulus* retinular cell.

The dark resistance decreases to about 80% in the test saline; the light-induced resistance change (ΔR_m) is small and soon becomes immeasurable. Also here the changes of ΔR_m and h_{max} are practically parallel. The determination of ΔR_m was less reliable (due to noise) than in *Limulus*.

The changes of the parameters were much less reversible in the after-period than in the case of *Limulus*.

The extracellular mass response of the whole retina under the influence of high external potassium was measured in earlier experiments (Stieve [14], *Eupagurus*, and Stieve and Wirth [15], *Astacus*). The changes are similar. During one hour in high potassium solution the excitability was not entirely abolished, h_{max} remained at 6 – 8%. The reversibility however was very good.

Potassium depolarization, 10% sodium

In five experiments (JA 17 – 21) the effect of high external potassium together with 10% of the normal sodium content (Table I, A 3) was simultaneously recorded in the *Astacus* retina intracellularly, with one microelectrode, and by extracellular measurement (mass response of the whole receptor cell layer). The resutls are shown in Tables V a, b and Figs 7 a, b. The results are similar as in the sodium-free potassium solution.

In the intracellularly recorded experiments (Table V a, Fig. 7 a) the PMP decreased sharply and reversed sign to +5 mV (half-time 9 min). The ReP disappeared (half-time 5 min). Again the total loss of excitability occurred some 10 min following the polarity change of the PMP. Again even when the PMP was reversed the ReP was still measurable.

With decreasing h_{max} (30%) t_{lat} was increased to 115%, t_{max} was decreased to 64% and t_2 to 35%. The shape-quotient (h_{max}/h_e) was strongly increased to over 300% after 60 min in the test saline. (As compared to the experiments without sodium t_{max} and t_2 are much more decreased when sodium is present. The shape-quotient is increased, while it is decreased without sodium.)

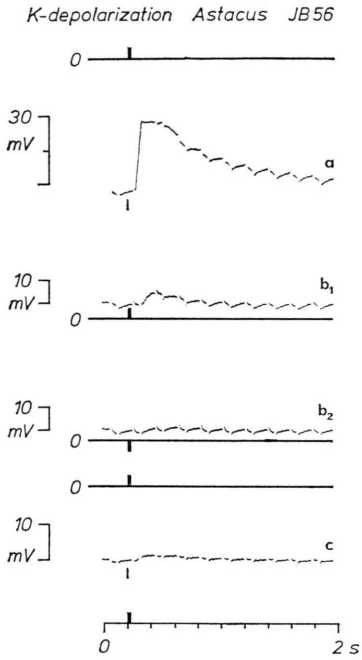

Fig. 6. Intracellularly recorded receptor potentials of an *Astacus* retinular cell and simultaneous measurement of the light-induced membrane resistance change ΔR. Light intensity ca. 5500 lx, stimulus duration τ 10 ms. a: in physiological saline; b_1: 55 min in test saline containing 212.3 mM/l potassium; b_2: 100 min in potassium test saline; c: 55 min again in physiological saline. JB 56, *Astacus* retinular cell.

The dark resistance did not change significantly, in contrast to the experiments where sodium was lacking. Again ΔR_m, which was hardly measurable due to noise, decreased parallelly to h_{max}.

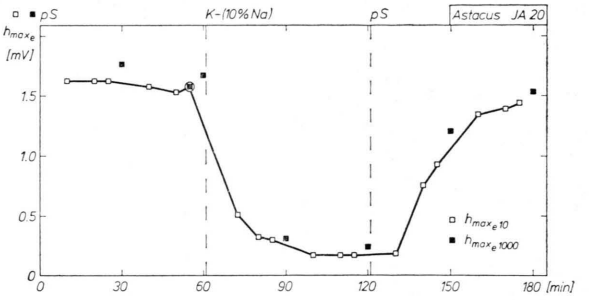

Fig. 7 b. Time course of the effect of potassium depolarization together with sodium deficiency on *Astacus* retina slice (same preparation from which the time course of the intracellular responses was recorded simultaneously, see Fig. 7 a). Extracellular measurement of $h_{max_{10}}$ [mV] and $h_{max_{1000}}$ [mV] *vs* time [min]. pS: perfusion with physiological saline; K (10% Na): perfusion with test saline. For further details see Fig. 7 a. JA 20, *Astacus* retina slice.

In the after-period h_{max} recovered to ca. 25%, and the PMP to ca. 20%. The light-induced resistance change reappeared, t_{max} and t_2 recovered, while t_{lat} remained unchanged.

The extracellularly recorded responses of the whole retina (Table V b, Fig. 7 b) showed similar results under the influence of the test saline. The ReP was decreased to 15% (half-time 8 min) and did not totally disappear. The shape-quotient was strongly increased, t_{lat} was increased, t_{max} and t_2 decreased. All changes showed good reversibility.

The main differences between extra- and intracellular measurement are: The decrease of h_{max} in the extracellular recording is smaller than that of h_{max} in the intracellular recording; t_2 is also less

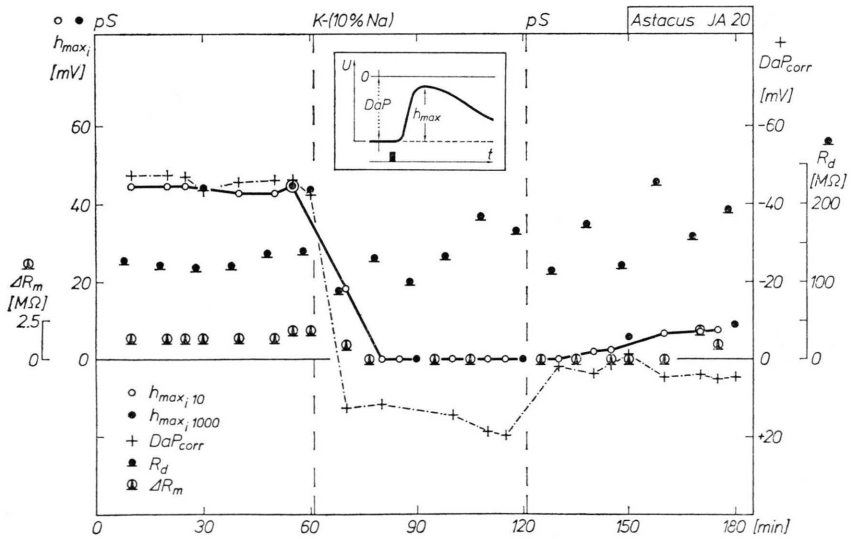

Fig. 7 a. Time course of the effect of potassium depolarization together with sodium deficiency on *Astacus* retinular cell. Intracellular measurement of $h_{max_{10}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV], R_d [MΩ] and ΔR_m [MΩ] *vs* time [min]. Light intensity ca. 20,000 lx. pS: perfusion with physiological saline; K (10% Na): perfusion with test saline. JA 20, *Astacus* retinular cell.

Table IV. Dependence of intracellularly recorded pre-stimulus membrane potential (PMP_{corr}), receptor potential and membrane resistance of *Astacus* retinular cells on 212 mM/l potassium added to the physiological saline (no sodium). Stimulus intensity ca. 5500 lx. JB 51−56, $n=5$.

1) $\tau=10$ ms

	PMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	−51 ± 6.4 mV	29 ± 5.1 mV	23 ± 2.3 ms	87 ± 7.9 ms	201 ± 27 ms	56 ± 17 MΩ	−1.6 ± 0.6 MΩ
values in test saline at							
h_{max} 50%	(−) 59 ± 4.7%	50%	125 ± 4.0%	87 ± 5.1%	64 ± 3.6%	86 ± 12%	−67 ± 11%
h_{max} 30%	(−) 18 ± 7.6%	30%	144 ± 8.1%	93 ± 17%	50 ± 4.3%	78 ± 11%	−22 ± 7.0%
h_{max} 0%	(+) 27 ± 19%	—	—	—	—	79 ± 11%	—
55 min in test saline	(+) 27 ± 13%	3 ± 3.0%	—	—	—	—	—
last value in test saline	(+) 23 ± 11%	—	—	—	—	—	—
55 min in physiological saline	(+) 11 ± 25%	11 ± 2.0%	151 ± 23%	85 ± 14%	71 ± 9.3%	78 ± 5.4%	—

2) $\tau=1000$ ms

	PMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	−50 ± 7.6 mV	32 ± 5.3 mV	172 ± 21 ms	21 ± 3.6 mV	14 ± 1.9 mV	1.5 ± 0.1	63 ± 21 MΩ	1.6 ± 0.6 MΩ	0.9 ± 0.4 MΩ
60 min in test saline	(+) 28 ± 16%	5 ± 4.6%	46%	6 ± 5.7%	—	81%	—	—	—
last value in test saline	(+) 22 ± 2.2%	0.2 ± 0.2%	50%	1.6 ± 1.6%	—	—	—	—	—
60 min in physiological saline	(−) 6 ± 11%	16 ± 2.9%	63 ± 7.6%	9 ± 5.7%	3 ± 2.0%	298 ± 102%	—	—	—

The half-time of the depolarization is $t_{1/2}=3.3\pm0.49$ min for h_{max} and $t_{1/2}=8\pm1.5$ min for the PMP. The signs in brackets indicate the polarity of the PMP.

Table V a. Dependence of intracellularly recorded pre-stimulus membrane potential (PMP_{corr}), receptor potential and membrane resistance of *Astacus* retinular cells on 192 mM/l potassium and 10% of the normal sodium concent in the physiological saline. Stimulus intensity ca. 20,000 lx. JA 17–21, $n=5$.

1) $\tau=10$ ms

	PMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	−49 ± 7.8 mV	42 ± 5.4 mV	16 ± 2.3 ms	98 ± 10 ms	316 ± 109 ms	66 ± 19 MΩ	−1.5 ± 0.6 MΩ
values in test saline at							
h_{max} 50%	(−) 34 ± 11%	50%	112 ± 2.8%	89 ± 9.3%	60 ± 6.6%	104 ± 21%	−49 ± 8.0%
h_{max} 30%	(+) 6 ± 14%	30%	115 ± 6.4%	64 ± 4.2%	35 ± 9.9%	112 ± 29%	−19 ± 11%
h_{max} 0%	(+) 9 ± 14%	—	—	—	—	107 ± 26%	—
55 min in test saline	(+) 18 ± 16%	—	—	—	—	—	—
last value in test saline	(+) 19 ± 17%	—	—	—	—	—	—
55 min in physiological saline	(−) 20 ± 30%	27 ± 7.1%	113 ± 7.3%	96 ± 11%	69 ± 13%	99 ± 14%	−13 ± 13%

2) $\tau=1000$ ms

	PMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	−50 ± 7.4 mV	47 ± 5.3 mV	160 ± 20 ms	29 ± 4.1 mV	24 ± 3.2 mV	1.7 ± 0.06	67 ± 19 MΩ	−2.2 ± 0.5 MΩ	+0.3 ± 0.5 MΩ
60 min in test saline	(+) 34 ± 20%	2 ± 1.5%	76 ± 13%	1.0 ± 0.7%	0.3 ± 0.3%	314 ± 180%	—	—	—
last value in test saline	(+) 34 ± 20%	3 ± 1.7%	67 ± 22% ($n=2$)	0.5 ± 0.24%	—	—	—	—	—
60 min in physiological saline	(−) 15 ± 24%	30 ± 9.2%	79 ± 19%	20 ± 8.2%	15 ± 6.0%	199 ± 44%	—	—	—

The half-time of the depolarization is $t_{1/2}=5.3\pm0.58$ min for h_{max} and $t_{1/2}=9\pm3.0$ min for the PMP. The signs in brackets indicate the polarity of the PMP.

Table V b. Dependence of extracellularly recorded receptor potentials of an *Astacus* retina slice (same preparation as in Table V a on 192 mM/l potassium and 10% of the normal sodium content in the physiological saline. Stimulus intensity ca. 20,000 lx. JA 17–21, $n=5$.

1) $\tau=10$ ms

	h_{max}	t_{lat}	t_{max}	t_2
reference value in physiological saline	1.4±0.25 mV	18±1.4 ms	111±21 ms	253±18 ms
values in test saline at				
h_{max} 50%	50%	128±6.8%	84±9.2%	87±6.2%
h_{max} 30%	30%	130±7.8%	78±9.9%	80±4.8%
55 min in test saline	14±1.3%	139±9.7%	79±12%	68±7.1%
55 min in physiological saline	83±5.1%	112±7.2%	95±11%	80±2.4%

2) $\tau=1000$ ms

	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
reference value in physiological saline	1.6±0.28 mV	160±60 ms	1.0±0.18 mV	0.7±0.15 mV	1.58±0.04
60 min in test saline	17±1.1%	99±25%	12±1.9%	0.6±0.6%	160±26%
60 min in physiological saline	82±3.7%	86±17%	68±3.6%	69±7.9%	125±7.7%

The half-time of the depolarization is $t_{1/2}=8\pm3.2$ min for h_{max}.

decreased in the extracellular measurement (80%) than the intracellular recording (35%).

The reversibility of all parameters is much better in the extracellular recording.

Discussion

Ouabain

Smith *et al.*[11] observed that the excitability of ventral photoreceptor cells of *Limulus* poisoned by ouabain was lost although the resting potential had decreased to only half of its original value. Our experiments confirm this observation. Since the explanation originally offered by Smith *et al.*[11], that the receptor potential was caused by the change in electrogenicity of an electrogenic ionic pump, is ruled out (Millecchia[16], Brown[17], Stieve *et al.*[7], Brown and Lisman[12]) there exist mainly two different possible explanations:

(1) The conductance change of the photoreceptor cell membrane does not occur when the visual cell is sufficiently poisoned by ouabain or sufficiently depolarized.

(2) The gradient of the ion species which determines the receptor potential (mainly Na^+) reaches zero earlier than the gradient determining the resting potential (mainly K^+).

At first the second explanation seemed to be plausible to us (Stieve[8]): The intracellular volume is very small compared with the extracellular volume (streaming saline). The height of the pre-stimulus membrane potential is determined mainly by the gradient of potassium ions due to a high intracellular and low extracellular concentration.

The height of the receptor potential is determined by other cations, mainly sodium (Stieve *et al.*[18], Stieve[1, 19]) having a high extracellular and low intracellular concentration. When, *e. g.*, Na^+ and K^+ are both diffusing along their concentration gradients at about the same rate, the Na^+ gradient is much faster decreased (intracellular Na^+ concentration soon increased due to small volume; extracellular K^+ concentration hardly changed due to great volume).

While this effect obviously may contribute to the loss of excitability, it seems to be not the decisive

factor, since our resistance measurements (Table II) show that receptor potential and conductance change cease to exist at the same time.

What are the reasons for the loss of ability for light-induced conductance changes? There are mainly three not necessarily exclusive possible causes:

(1) As in the nerve membrane the conductance change of the visual cell membrane can no longer occur when the membrane potential is below a certain value.

(2) Poisoning of the active Na^+-transport by ouabain leads, as suggested by Brown and Lisman [12], to intracellular accumulation of sodium ions, which in turn leads to an increase in intracellular Ca^{2+}. This increase in intracellular calcium causes the progressive loss of light-induced conductance change.

(3) There is a direct action of ouabain – or of the metabolic energy used for the active transport – on the permeability changing system of the visual cell membrane.

Baumann and Mauro [20] performed experiments in the photoreceptor of the lateral eye of *Limulus* and the drone photoreceptor where the ability for conductance changes disappears fast when there is no oxygen at the visual cell – by applying a N_2 atmosphere. When oxygen is added again the dark potential is regained earlier than the ability for conductance change. These experiments may indicate a direct need of the cell membrane for metabolic energy. It is conceivable that the activity, or the intact state, of the sodium/potassium ATPase, is necessary for the membrane to undergo light-induced conductance changes.

In the ouabain experiments the loss of excitability occurs at much higher membrane potentials than in the case of the potassium depolarization. Either ouabain has a direct action on the permeability system or, since the ouabain depolarization occurs much more slowly than the potassium depolarization, the influence of the decreased membrane potential has sufficient time to develop, so that the blockage occurs at higher membrane potentials. Since normally depolarications of up to 50% alone do not block the excitability of visual cells, an indirect influence of ouabain, mediated via the membrane potential, is not sufficient to explain the findings. However a loss of excitability via an increase of intracellular Ca^{2+}, as suggested by Brown and Lisman [12], provides a reasonable and perhaps sufficient explanation for the observed ouabain action. Whether additionally the decrease in membrane potential has a harmful influence on excitability cannot yet be decided.

In the early phase of the ouabain treatment there is always an increase of the pre-stimulus membrane potential and in most cases of the receptor potential. Probably the early action (or low concentration) of ouabain on the membrane influences the membrane permeability. This effect is not necessarily an action on the ionic pump, but could be a direct action on other components of the membrane, for instance the dark channels. The mechanism of this effect was not investigated further.

Depolarization by high external potassium concentration

An increase in intracellular Ca^{2+} might perhaps be a sufficient explanation for the observed action of ouabain on the visual cell. However, it is not a probable explanation for the loss of light-induced conductance changes due to potassium depolarization expectially since the half time, $t_{1/2}$, for the decrease in h_{max} does not differ under conditions of no or 10% of normal external sodium. Here the obvious explanation seems to be that a certain minimal (lasting) membrane potential is a necessary prerequisite for the ability to light-induced conductance changes of the photosensoric membrane. Since inexcitability occurs in the presence of 10% external sodium with the same efficiency and speed as when there is no sodium present, the extracellular sodium concentration is apparently not responsible for this effect.

The experiments indicate a direct influence of the membrane potential on the ability of the cell membrane to undergo light-induced permeability changes, but the action of depolarization develops much slower than in the case of the axon membrane. Probably the membrane potential is necessary to create and sustain an ordered structure of membrane constituents of dipole character. This order seems to be essential for the ability to produce conductance changes.

The existence of small but still measurable receptor potentials in depolarized visual cells in the first minutes of depolarization with already reversed (in sign) pre-stimulus membrane potential indi-

cates that at least in certain areas of the cell membrane there is still an inward flux (and gradient) of positive ions, most probably sodium ions. As in intracellular recordings inexcitability occurs some 10 min after total depolarization, one could assume that during this time the test saline has succeeded to reach also these areas of the cell membrane. The fact that in extracellular recordings the excitability is never totally abolished (h_{max} remains at a level of 5 – 15%) cannot be explained by slow replacement of the salines. Perhaps part of the unpenetrated cells are inaccessible to the potassium – rich saline, or they can better resist the strain, or active transport helps to produce some ionic gradient necessary for the still measurable mass response.

The fact that experimental conditions decrease the intracellular response to zero, while a small response is still recorded extracellularly applies to treatments by many substances besides ouabain and potassium: For instance treating the retina with 1 mM/l X537A causes total loss of excitability in most of the intracellular recordings, whereas it causes a diminution of the receptor potential to ca. 5% in the extracellular recordings (Stieve and Bruns [21]). Treatment of the retina with calcium-free solution, containing 1 mD/l EDTA or EGTA, causes, in many cases, irreversible loss of excitability when recorded intracellularly but always reversible loss of excitability in the extracellular recorded case (Stieve [8]).

Some final speculations

The experiments with high external potassium described here show that the ability of the visual cell membrane to exert light-induced conductance changes depends upon the membrane potential, but the action of the decrease of potential is delayed by several minutes.

The membrane potential could affect the order or orientation of some dipolar membrane constituents. When the electrical field in the membrane is changed, by lowering or reversal of the membrane potential, the order is no longer sustained in the same way and the molecules can change their orientation. The original order might be necessary for the ability of the membrane to undergo conductance changes.

In the first line those molecules forming the ionic channels in the visual cell membrane could be responsible for the dipole orientation effect. In artificial lipid bilayer membranes channel-forming substance are known which change their orientation due to the membrane potential. Alamethicin is such a substance a charged part of which is believed to be drawn into the lipid layer of the membrane by the membrane potential (Mueller [23], Boheim and Kolb [24]).

Another possibility would be that the proper orientation of the rhodopsin molecules could be necessary for conductance change. We think it not very probably that a disorientation of the rhodopsin molecules is involved, since the early receptor potential, which is believed to be a signal coming from a change in dipole moment of rhodopsin molecules, can be recorded for up to 2 hours without decrement or disruption in totally depolarized visual cells (Dahl [22]).

Perhaps the light-induced conductance change in the visual cell membrane is additionally in some way more directly energy dependent or dependent on a functional sodium-potassium ATPase.

We want to thank R. Backbier, H. Breuer and J. Appelhans for technical help, K. Hartung for many discussions and critical reading of the manuscript and E. Brentgens for help with the manuscript.

This study was supported by the DFG under the research program SFB 160.

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