

The Effect of Changed Extracellular Calcium and Sodium Concentration on the Electretinogram of the Crayfish Retina

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Z. Naturforsch. **35 c**, 308–318 (1980); received December 17, 1979

Astacus Retina, Electretinogram, Extracellular Calcium and Sodium Concentration

The electretinogram (ERG) of the isolated retina of the crayfish *Astacus leptodactylus* evoked by strong 10 ms light flashes at constant 5 min intervals was measured while the retina was continuously superfused with various salines which differed in Ca^{2+} - and Na^{+} -concentrations. The osmotic pressure of test- and reference-saline was adjusted to be identical by adding sucrose.

Results:

1. Upon raising the calcium-concentration of the superfusate in the range of 20–150 mmol/l (constant Na^{+} -concentration: 208 mmol/l) the peak amplitude h_{max} and the half time of decay t_2 of the ERG both decrease gradually up to about 50% in respect to the corresponding value in reference saline.

2. The recovery of the ERG due to dark adaptation following the “weakly light adapted state” is greatly diminished in high external $[\text{Ca}^{2+}]_{\text{ex}}$.

3. Lowering the external calcium-concentration (10 → 1 mmol/l) causes a small increase in h_{max} and a strong increase of the half time of decay t_2 (about 180%).

Upon lowering the calcium concentration of the superfusate to about 1 mmol/l by 1 mmol/l of the calcium buffer EDTA, a slowly augmenting diminution of the ERG height h_{max} occurs. However, a strong retardation of the falling phase of the ERG characterized by an increase in t_2 occurs quickly. Even after 90 min stay in the low calcium saline the retina is still not inexcitable; h_{max} is 5–10% of the reference value. The diminution of h_{max} occurs about six-fold faster when the buffer concentration is raised to 10 mmol/l EDTA.

4. Additional lowering of the Na^{+} -concentration (208 → 20.8 mmol/l) in a superfusate with a calcium concentration raised to 150 mmol/l causes a strong reduction of the ERG amplitude h_{max} to about 10%.

5. In a superfusate containing 1 mmol/l calcium such lowering of the sodium concentration (208 → 20.8 mmol/l) causes a diminution of the ERG height to about 40% and the shape of the ERG to become polyphasic; at least two maxima with different time to peak values are observed.

Interpretation:

1. The similarity of effects, namely raising external calcium concentration and light adaptation on the one hand and lowering external calcium and dark adaptation on the other hand may indicate that the external calcium is acting on the adaptation mechanism of the photoreceptor cells, presumably by influencing the intracellular $[\text{Ca}^{2+}]$.

2. The great tolerance of the retina against Ca^{2+} -deficiency in the superfusate might be effected by calcium stores in the retina which need high Ca^{2+} -buffer concentrations in the superfusate to become exhausted.

3. In contrast to the *Limulus* ventral nerve photoreceptor there does not seem to be an antagonistic effect of sodium and calcium in the crayfish retina on the control of the light channels.

4. The crayfish receptor potential seems to be composed of at least two different processes. Lowering calcium- and lowering external sodium-concentration both diminish the height and change the time course of the two components to a different degree. This could be caused by influencing the state of adaptation and thereby making the two maxima separately visible.

Introduction

The membrane potential of photoreceptors is caused by the different distribution of ions across the cell membrane and the specific permeability of the cell membrane for these ions. In invertebrate photoreceptors the light-induced change of the membrane

potential, the receptor potential, is mainly due to an increase in sodium conductance. The dark potential is mainly determined by the gradient of potassium ions (Millecchia and Mauro [1, 2], Fulpius and Baumann [3], Brown *et al.* [4], Stieve [5], Stieve and Wirth [6], Wulff [7], Brown [8]).

Calcium ions which may also contribute to the generation of the receptor potential (Brown *et al.* [4] and Brown [9]) seem to play an important role mainly in the adaptation processes by changing the

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0341-0382/80/0300-0308 \$ 01.00/0



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sensitivity of the photoreceptor cell (Lisman and Brown [10], Brown [9], and Stieve [11, 12]). The former have shown this in experiments with *Limulus* ventral nerve photoreceptors. It could also be shown in *Eupagurus* (Stieve [13]), *Astacus* (Stieve and Wirth [6], Stieve and Hanani [14]) and in *Balanus* (Hanani and Hillman [15]). According to the hypothesis of Lisman and Brown [10] the sensitivity of the *Limulus* photoreceptor is controlled in adaptation via the level of the intracellular calcium ion concentration. Stimulation by light causes an increase in $[Ca^{2+}]_{in}$ which in turn causes a reduction of the sensitivity of the photoreceptor (Brown and Blinks [16], Fein and Charlton [17], Maaz [18]).

The effect of lowering the extracellular calcium concentration $[Ca^{2+}]_{ex}$ has been studied in the past more frequently (Brown *et al.* [4], Fulpus and Baumann [3], Lisman and Brown [10], Millecchia and Mauro [1], Brown and Mote [19]).

A discrepancy was observed when $[Ca^{2+}]$ (and $[Mg^{2+}]$) was lowered to the micromolar range: *Limulus* and *Eupagurus* photoreceptors became inexcitable, whereas *Astacus* retinas stayed excitable for several hours. Raising of $[Ca^{2+}]_{ex}$ leads in *Eupagurus* to a diminution or abolition of the light response but has only been tested with salines of lowered sodium concentration in contrast to the experiments with *Astacus*.

In this paper the influence of changed external calcium concentration on the electroretinogram (ERG) of the isolated crayfish retina and its changes by adaptation are described. The aim of this investigation was to understand the discrepancies of the effect of very low Ca^{2+} - and Mg^{2+} -concentrations between *Astacus* on the one hand and *Eupagurus* and *Limulus* on the other hand. In addition it was tested whether the diminution of the light response in raised $[Ca^{2+}]_{ex}$ is due to the calcium increase alone or whether the reduction in sodium simultaneously has a substantial influence.

The Ca^{2+} -concentration of the superfusate was varied between 150 mmol/l and 1 nmol/l by adding the Ca^{2+} -buffer EDTA. The concentrations of the other ions of the "physiological" Van Harreveld solution were kept constant or in other experiments the sodium concentration was additionally lowered to 10%. When the osmotic pressure of the test saline was higher than that of the Van Harreveld solution the osmotic pressure of the reference saline was adjusted by adding sucrose.

Materials and Methods

Object and Preparation

Retinas from compound eyes of the crayfish *Astacus leptodactylus* Eschholz were isolated in a similar way as described elsewhere (Stieve and Wirth [6]). In the dissection procedure the cornea, the crystalline cones and most part of the optic ganglia were removed.

Solutions

A modified Van Harreveld solution (Van Harreveld [20]) without Mg^{2+} -ions is used as physiological saline (see Table I). The pH-value of the solution is adjusted to 7.5. The reference saline is this physiological saline with its osmotic pressure adjusted to be identical with the test saline by adding sucrose.

The ionic composition of the test salines by which the retina is superfused is also shown in Table I.

Procedure of experiments

The whole isolated retina is placed with its proximal side on a Ag-AgCl-electrode which is situated in the lower part of a plexiglass vessel (Fig. 1). The retina is fastened by a black sylgard-ring which encloses the retina tightly. In the upper part of the vessel the reference electrode is situated, which is in contact with the streaming solution.

The retina is stimulated in 5 min intervals by constant white flashes of 10 ms duration of a Xenon

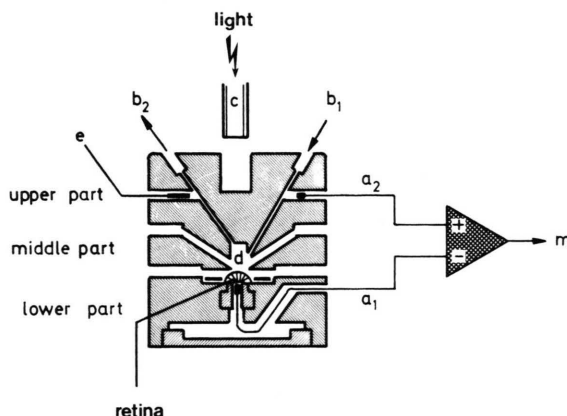


Fig. 1. Diagram of the plexiglass-vessel; a_1 , measuring electrode (Ag-AgCl); a_2 , reference electrode (Ag-AgCl); b_1 , solution inlet; b_2 , solution outlet; c , fiber optic; d , inner test chamber, volume approx. 0.1 cm³; e , temperature sensor; m , measurement equipment.

Table I. Ionic composition in mmol/l of the salines used in the experiments.

		Osmotic pressure [mosmol]	NaCl	KCl	CaCl ₂	Sucrose	Tris ^a	Hepes ^b	EDTA ^c	NaOH
1	1 mmol/l Ca ²⁺	430	207.8	5	1	27	10	—	—	—
PS ⁺	10 mmol/l Ca ²⁺	430	207.8	5	10	—	10	—	—	—
2	20 mmol/l Ca ²⁺	465	207.8	5	20	—	10	—	—	—
3	50 mmol/l Ca ²⁺	570	207.8	5	50	—	10	—	—	—
4	100 mmol/l Ca ²⁺	700	207.8	5	100	—	10	—	—	—
5	150 mmol/l Ca ²⁺	880	207.8	5	150	—	10	—	—	—
6	low Na ⁺ high Ca ²⁺	880	20.78	5	150	374	10	—	—	—
7	low Ca ²⁺	430	207.8	5	10 ⁻⁶	29	—	10	1	—
8	low Ca ²⁺ low Na ⁺	430	20.78	5	10 ⁻⁶	403	—	10	1	—
9	low Ca ²⁺	430	177.8	5	10 ⁻⁶	50	—	10	10	30

⁺ PS = physiological saline (Van Harreveld solution).

^a Tris (hydroxymethyl) aminomethane.

^b [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid].

^c ethylene – diamine – tetra – acetic acid.

lamp using fiber optics. The intensity of the stimulating light at 543 nm is about $12 \text{ mW} \cdot \text{cm}^{-2}$ which corresponds to about $3 \times 10^{16} \text{ photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$. The spectral sensitivity of *Astacus leptodactylus* has its maximum at 565 nm (Stieve, Gerhards [21]). Half-saturation is reached when the light intensity is reduced to about 0.1% of this intensity.

Due to the light flash an electroretinogram (ERG) is evoked which is the sum of the photoreceptor currents of the single visual cells. This extracellular measurement records the potential drop over the extracellular shunt resistance parallel to the long

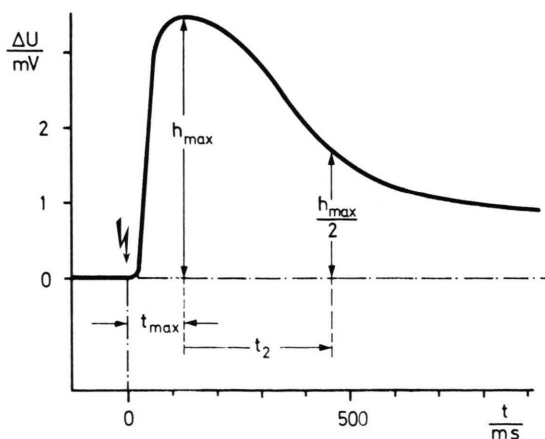


Fig. 2. Schematic drawing of the three ERG parameters used: h_{max} , t_{max} and t_2 .

axes of the retinulas (Stieve *et al.* [22]). Therefore the magnitude of the voltage signal depends on the ratio of shunt resistance (R_S) to membrane resistance (R_M). From the ratio of extra- and intracellular response amplitudes this ratio is estimated to be in the order of 1:10. Any change in the ratio of R_S/R_M will influence the height of the ERG. Probably R_S/R_M will be altered when the osmotic pressure of the superfusate is changed.

The temperature of the superfusate is kept constant at 15 °C. The perfusion rate of the test chamber is 1 ml/min and the half-time of the saline's exchange in the experimental chamber takes about 40 s after the switch to a new saline. The retina was first superfused with the reference saline during a pre-period of 30 min. Then the reference saline is exchanged by the test solution which is a saline of varied ionic composition. The superfusion of the retina by the test solution lasted in general 35 min and was followed by an after-period in the reference saline to test the reversibility of the light response. For each concentration 4–5 experiments were carried out.

Evaluation of the experiments

The following parameters of the ERG were measured (see Fig. 2):

h_{max} (mV), the maximum height of the ERG;

t_{max} (ms), time to peak: the time from the beginning of the stimulus to h_{max} ;
 t_2 (ms), the half time of the decay in which the ERG decreases from h_{max} to $h_{max}/2$.

h_{max} , t_{max} and t_2 were normalized (%) in respect to the reference value of the last ERG recorded at the end of the pre-period in reference saline.

Results

Raising $[Ca^{2+}]_{ex}$ (10 → 20, 50, 100, 150 mmol/l)

The effect of raised $[Ca^{2+}]_{ex}$ was studied in 4 sets of experiments one for each concentration tested. Each set contained 4–5 experiments. The results are shown in Figs 3 and 4 and summarized in Table IV and Figs 5–7. Table III shows the evaluation of one set of experiments (100 mmol/l Ca^{2+}).

In a number of experiments the influence of *high osmotic pressure* alone on the ERG was tested. (Table II, see also Fig. 4.) Increasing the osmotic pressure results in a gradual reduction of the ERG height h_{max} , an increase in time to peak t_{max} and a shortening of the decrease time t_2 . The effect of high osmolality on the ERG slowly increases in time. In the experiments described in the following the osmotic pressure of the reference saline is always adjusted to be identical with the test saline.

Raising the external Ca^{2+} -concentration causes a gradual diminution of the ERG height h_{max} . The effect of high $[Ca^{2+}]_{ex}$ increases in time – the more, the higher the Ca^{2+} -concentration is – however, even in very high Ca^{2+} -concentrations (150 mmol/l) the retina does not become inexcitable within 20 min (Figs 3 and 4).

The time to peak t_{max} also decreases with increasing $[Ca^{2+}]_{ex}$. The decline of the ERG is accelerated with increasing Ca^{2+} ; t_2 decreases. There

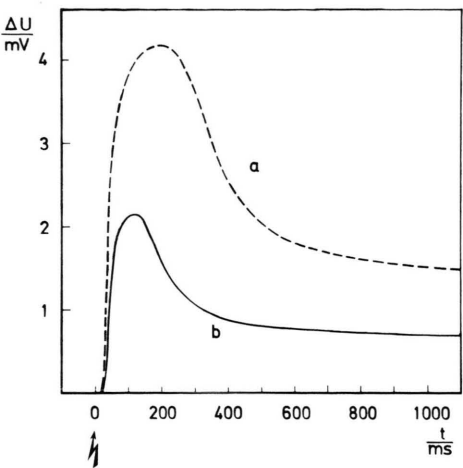


Fig. 3. Change of the ERG of the isolated *Astacus* retina, due to raising the external calcium concentration to 150 mmol/l. a, last value in the reference saline of the pre-period; b, after 20 min stay of the retina in a solution with 150 mmol/l Ca^{2+} .

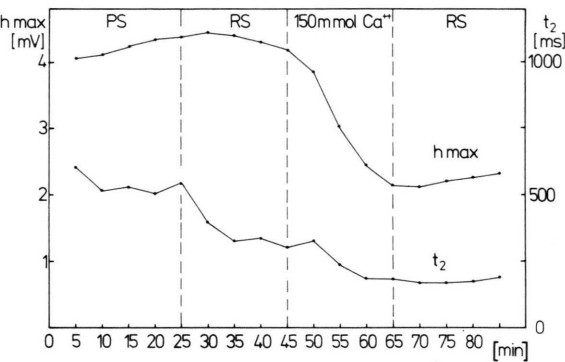


Fig. 4. Time course of the effect of raised external calcium concentration (150 mmol/l). PS, superfusion with physiological saline (Van Harreveld solution); RS, superfusion with reference saline (= physiological saline osmotically adjusted to the test saline); 150 mmol Ca^{2+} – superfusion with the test saline.

Table II. The effect of raising the osmotic pressure by adding sucrose to the physiological saline.

	Osmotic pressure [mosmol]			
	430 ^a after 35 min	700 after 35 min	800 after 35 min	880 after 20 min
h_{max}	2.9 ± 0.5 mV	71.8 ± 7.9%	65.4 ± 3.7%	54.6 ± 2.5%
t_{max}	104.1 ± 5.0 ms	136 ± 12 %	129.0 ± 5.3%	161.5 ± 9.8%
t_2	418 ± 114 ms	82.4 ± 3.9%	82.1 ± 4.1%	88.2 ± 7.2%

^a The mean reference value in physiological saline with normal osmotic pressure ($n=20$).

Table III. Evaluation of one set of experiments ($n=5$): the influence of raised external calcium-concentration (100 mmol/l).

	Pre-period: last value in reference saline	Test-period: after 35 min in 100 mmol/l $[Ca^{2+}]_{ex}$	After-period: after 35 min in reference saline
h_{max}	0.55 ± 0.07 mV	$58.1 \pm 5.9\%$	$104.1 \pm 4.3\%$
t_{max}	115 ± 15 ms	$65.6 \pm 4.1\%$	$86.5 \pm 2.6\%$
t_2	213 ± 17 ms	$53.3 \pm 6.0\%$	$76.8 \pm 4.8\%$

are indications that the effect of raising $[Ca^{2+}]_{ex}$ on the ERG saturates at about 150 mmol/l (Figs 5–7, Table IV). Raising the $[Ca^{2+}]_{ex}$ influences h_{max} and t_2 in the same direction as raised osmotic pressure whereas it influences t_{max} in the opposite direction (Table II and Table IV).

The reversibility in the after-period is the better the smaller the raise in external calcium is. Even after 20 min in 150 mmol/l Ca^{2+} h_{max} has recovered from 50% ($\pm 3\%$) to 72% ($\pm 6\%$) at the end of the 20 min after-period in reference saline.

In different sets of experiments a period of about 1 h dark adaptation was followed after stimulation in regular 5 min intervals as usual. The first ERG

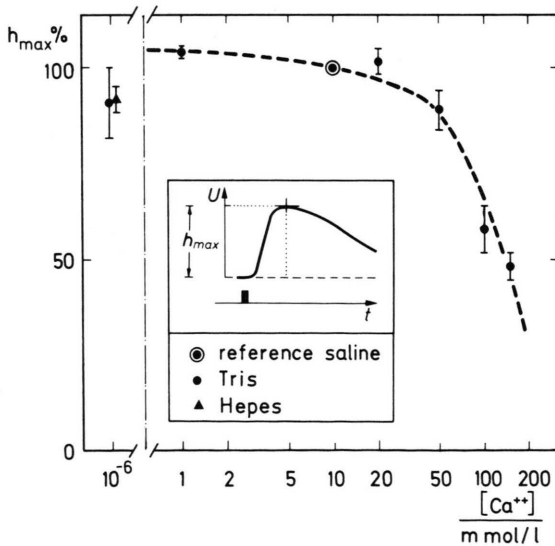


Fig. 5. The maximum height of the ERG h_{max} in % of the reference value \odot recorded in reference saline (10 mmol/l Ca^{2+}) versus extracellular calcium concentration. There are two values for 10^{-6} mmol/l Ca^{2+} of the two sets of experiments which were carried out with Tris and Hepes as buffer, resp. The error bars indicate the standard errors of the mean. Reference value $h_{max} \cong 2.23 \pm 0.55$ mV; $n = 29$.

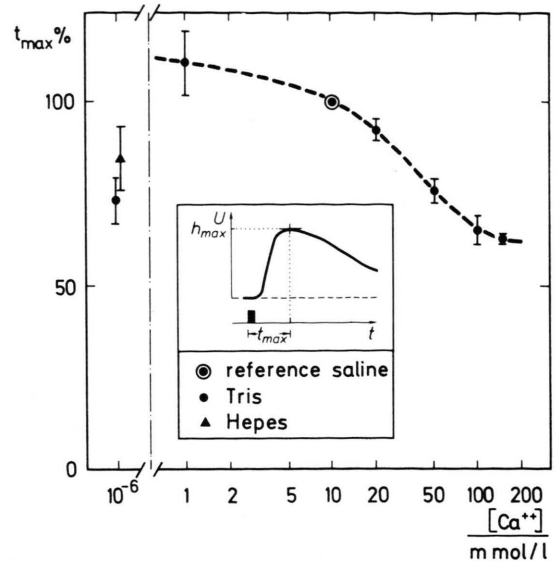


Fig. 6. The time to peak t_{max} in % of the reference value \odot recorded in reference saline (10 mmol/l Ca^{2+}) versus extracellular calcium concentration. Further details as in Fig. 5. Reference value $t_{max} \cong 115.7 \pm 7.5$ ms; $n = 29$.

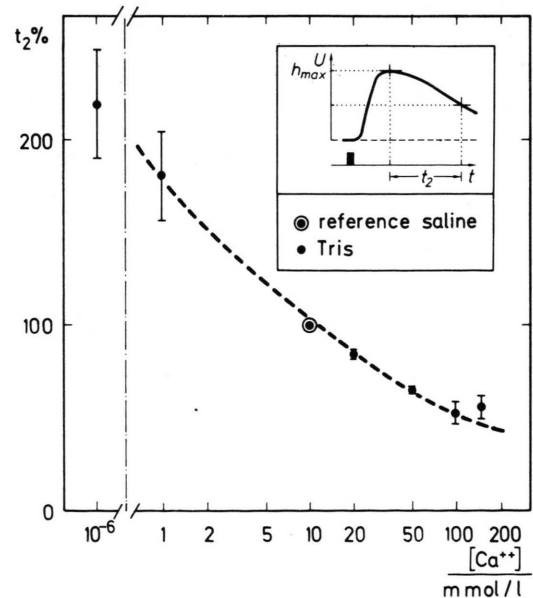


Fig. 7. The half time of decay t_2 in % of the reference value \odot recorded in reference saline (10 mmol/l Ca^{2+}) versus extracellular calcium concentration. In the set of experiments with 10^{-6} mmol/l Ca^{2+} and Hepes as buffer t_2 is > 1000 ms and therefore no longer measurable in our records. Further details as in Fig. 5. Reference value $t_2 \cong 351 \pm 88$ ms; $n = 29$.

Table IV. Tabular summary of the experiments with raised and lowered external calcium-concentration. For each concentration the values are averaged from one set of experiments and normalized (in per cent) to the reference value. The 100% value at 10 mmol/l Ca^{2+} (reference saline) is the average value of all reference salines ($n=29$). All values have been measured after 35 min stay of the retina in the test saline except the set of experiments with 150 mmol/l Ca^{2+} , which has been measured after 20 min stay.

$[Ca^{2+}]$ mol/l	10^{-9} Tris	Hepes	10^{-3}	10^{-2}	$2 \cdot 10^{-2}$	$5 \cdot 10^{-2}$	10^{-1}	$1.5 \cdot 10^{-1}$
osmotic pressure [mosmol]	430	430	430	430	465	570	700	880
number of experiments	3	4	4	29	4	5	5	4
	%	%	%	100%	%	%	%	%
h_{max}	91.2 ± 9	92.0 ± 3.2	104.0 ± 1.5	$\triangleq 2.23 \pm 0.55$ mV	101.8 ± 3.1	89.2 ± 5.1	58.1 ± 5.9	49.5 ± 3.5
t_{max}	73.3 ± 6.2	84.7 ± 8.8	110.1 ± 8.8	$\triangleq 115.7 \pm 7.5$ ms	92.4 ± 3.0	75.8 ± 3.4	65.6 ± 4.1	63.6 ± 1.1
t_2	219 ± 29	>1000 ms	182 ± 24	$\triangleq 351 \pm 88$ ms	85.0 ± 2.4	65.5 ± 1.9	53.3 ± 6.0	56.3 ± 6.2

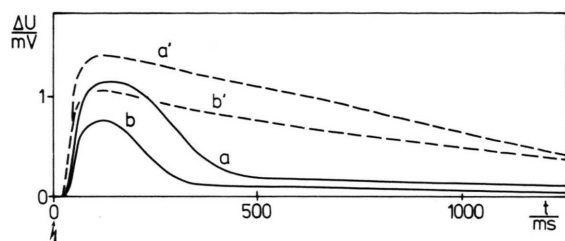


Fig. 8. Change of the ERG after dark adaptation in reference saline and in raised external calcium concentration (50 mmol/l). a, last value in the reference saline after stimulation in regular 5 min intervals; a', the first ERG recorded after 1 h darkness in reference saline containing 10 mmol/l Ca^{2+} ; b, after 35 min in 50 mmol/l Ca^{2+} ; stimulation in regular 5 min intervals; b', the first ERG recorded after 1 h darkness in the test saline containing 50 mmol/l Ca^{2+} .

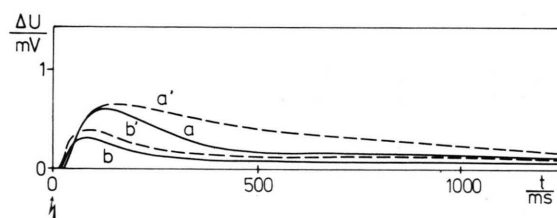


Fig. 9. Change of the ERG after dark adaptation in reference saline and in raised external calcium concentration (150 mmol/l). a, after 20 min in reference saline after stimulation in regular 5 min intervals; a', the first ERG recorded after 80 min darkness in reference saline containing 10 mmol/l Ca^{2+} ; b, after 20 min in 150 mmol/l Ca^{2+} after stimulation in regular 5 min intervals; b', the first ERG recorded after 80 min darkness in the test saline containing 150 mmol/l Ca^{2+} .

evoked at the end of this dark period was compared with the one immediately before dark adaptation in a steady state of weak light adaptation (Figs 8 and 9).

In reference saline and in test saline with $[Ca^{2+}]_{ex}$ up to 50–100 mmol/l the dark adapted response is much greater and the decrease much slower than the weakly light adapted response (Fig. 8). In very high calcium-concentration (150 mmol/l) the dark adapted response caused by prolonged stay in darkness (80 min) is almost identical with the weakly light adapted one (Fig. 9). The weakly light adapted response evoked by stimulation in regular 5 min intervals becomes smaller and shorter with increasing

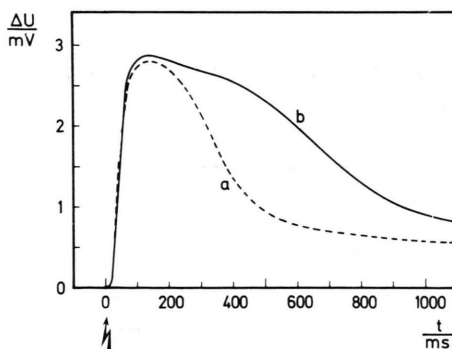


Fig. 10. Change of the ERG of the isolated *Astacus* retina due to lowering the external calcium concentration to 1 mmol/l. a, last value in physiological saline; b, after 35 min stay of the retina in a saline with 1 mmol/l Ca^{2+} .

Fig. 11. Time course of the effect of lowered external calcium concentration (1 mmol/l). PS, superfusion with physiological saline (Van Harreveld solution). 1 mmol Ca^{2+} — superfusion with the test saline.

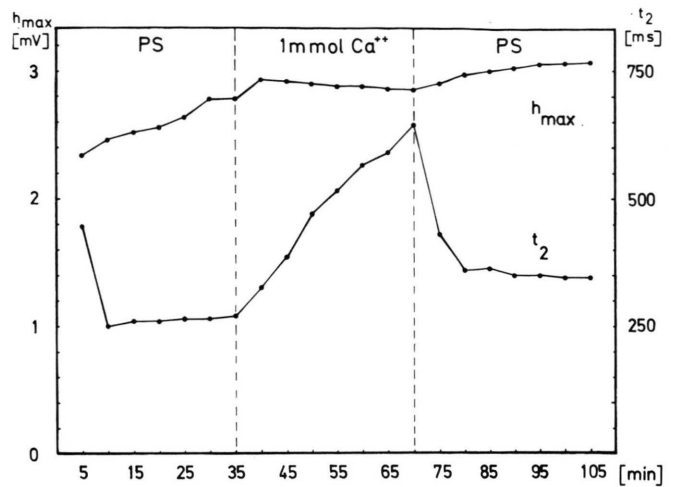
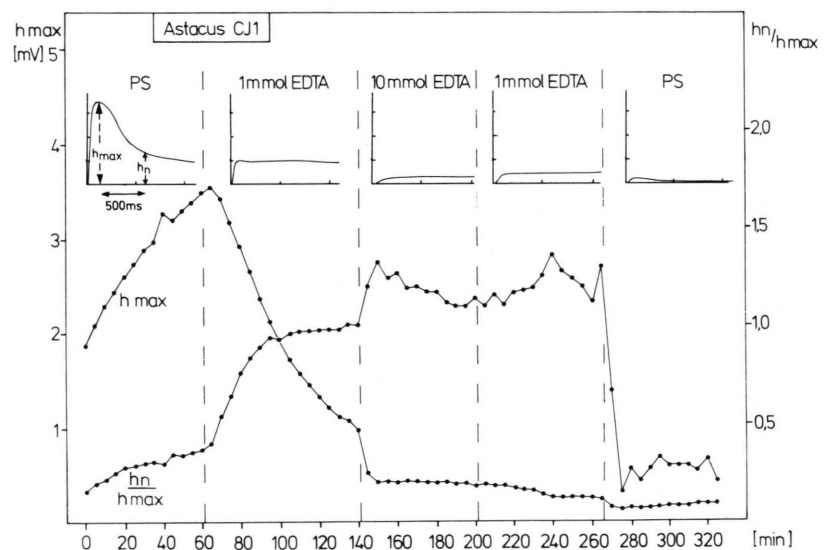


Fig. 12. Time course of the effect of lowering the external calcium ion concentration to about 1 nmol/l consecutively by adding 1 mmol/l EDTA, 10 mmol/l EDTA and again 1 mmol/l EDTA. h_n is the ERG amplitude 500 ms after h_{max} (see inset). The quotient h_n/h_{max} shows the change of the ERG shape. If the quotient h_n/h_{max} is close to 1 the shape of the ERG is "rectangular".



calcium-concentration starting from the lowest calcium-concentration tested.

The influence of lowering the external calcium-concentration

a) 10 mmol/l \rightarrow 1 mmol/l

The results of this set of experiments ($n = 4$) are shown in Figs 5, 6, 7, 10 and 11, and Table IV.

Lowering $[Ca^{2+}]_{ex}$ to 1 mmol/l increases h_{max} and t_{max} slightly but retards the decay of the ERG (i. e. t_2 is increased) very strongly. Whereas h_{max} reaches a stationary height soon after the switch to the 1 mmol/l

calcium superfusate, t_2 grows steadily during the entire 35 min of the test period (see Fig. 11). The reversibility is quite good.

b) 10 mmol/l \rightarrow 1 nmol/l by adding the Ca^{2+} -buffer EDTA in different concentrations

The results are shown in Figs 12, 13 and 16 and Table IV.

Lowering the external calcium- (and magnesium-) concentration very strongly by adding 1 mmol/l EDTA does not abolish the ERG but reduces the height of the ERG slowly. After 35 min in this test saline h_{max} is 92% ($\pm 3\%$) of the reference value

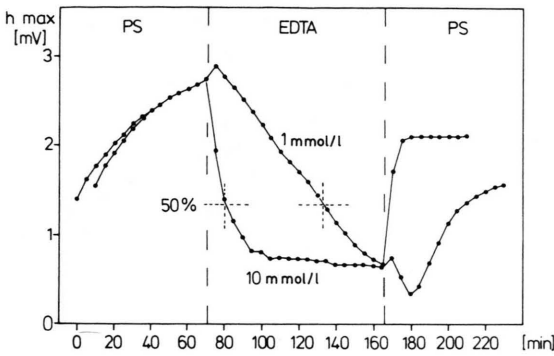


Fig. 13. Time course of two different experiments with test salines containing 1 nmol/l Ca^{2+} one with 1 mmol/l EDTA and the other one with 10 mmol/l EDTA. The time needed to reduce h_{max} to 50% is about $\frac{1}{3}$ in 10 mmol/l EDTA. 10 mmol/l EDTA: h_{max} is 50% after 11 min; 1 mmol/l EDTA: h_{max} is 50% after 63 min. Further details as in Fig. 12.

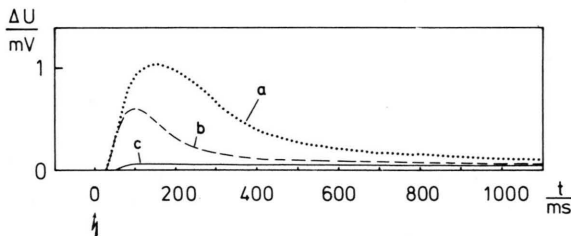


Fig. 14. Change of the ERG of the isolated *Astacus* retina due to raising the external calcium concentration to 150 mmol/l (b) and additional lowering the sodium concentration to 20.8 mmol/l (c). a, last value in the reference saline of the pre-period; b, after 20 min in a saline with 150 mmol/l Ca^{2+} and normal sodium concentration (208 mmol/l); c, after 20 min in a saline with 150 mmol/l Ca^{2+} and 20.8 mmol/l Na^+ .

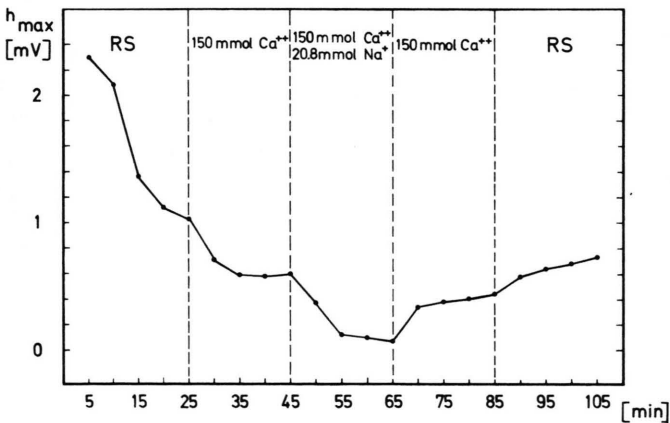


Fig. 15. Time course of the effect of raised external calcium concentration (150 mmol/l) and additional lowered external sodium concentration (20.8 mmol/l) on the peak amplitude h_{max} . RS, superfusion with reference saline; 150 mmol Ca^{2+} , superfusion with the test saline (150 mmol/l Ca^{2+} , normal $[Na^+]$ (208 mmol/l); 150 mmol Ca^{2+} , 20.8 mmol Na^+ , superfusion with the test saline containing 150 mmol/l Ca^{2+} and 20.8 mmol/l Na^+ .

(Table IV) and after 1.5 h h_{max} is lowered to 25–30% (Fig. 13).

The decay of the ERG following the maximum is slowed down tremendously to such a degree that t_2 is > 1000 ms and therefore no longer measurable in our records (Fig. 16, curve b).

In the photoreceptors of *Limulus* and *Eupagurus* such a strong reduction of external $[Ca^{2+}]$ and $[Mg^{2+}]$ turns the receptor to become inexcitable. To test whether the Ca^{2+} -buffer capacity used is sufficient to reduce the extracellular calcium concentration in the vicinity of the photosensory membranes strongly we applied in another set of 3 experiments a test saline with 10-fold the EDTA concentration of the former set (Figs 12 and 13).

In 10 mmol/l EDTA the reduction of h_{max} occurs considerably faster than in 1 mmol/l EDTA (Fig. 13). The influence of the calcium-buffer capacity can also be demonstrated when the two EDTA concentrations are applied consecutively (Fig. 12). Figs 12 and 13 show that changing solely the buffer concentration leads to a conspicuous acceleration of the h_{max} decrease.

The retardation of the ERG decay characterized by the quotient h_n/h_{max} (h_n = potential height 500 ms after h_{max}) reaches in 1 mmol/l EDTA a stable value (within 20–30 min) earlier than h_{max} whereas h_{max} increases during the test period (Fig. 12).

The influence of raised external calcium

(10 → 150 mmol/l) and additional lowering of external sodium (208 → 20.8 mmol/l)

The results of this set of experiments are shown in Figs 14 and 15.

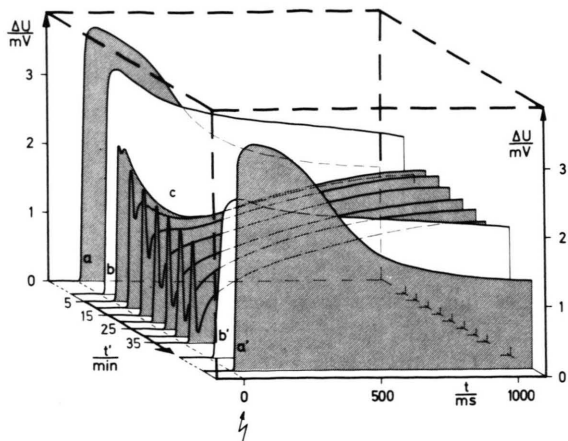


Fig. 16. The change of the ERG in lowered external calcium concentration (about 1 nmol/l) by 1 mmol/l EDTA and additional lowered external sodium concentration (20.8 mmol/l) depending on the time course of the experiment. x , axis: recording time; z , axis: experimental time. a , last record in physiological saline; b , after 35 min stay in 1 nmol/l Ca^{2+} and normal sodium concentration (208 mmol/l); c_{1-7} , the following ERGs recorded in 5 min intervals in a saline with 1 nmol/l Ca^{2+} and 20.8 mmol/l Na^+ ; b' , after 35 min stay of the retina in 1 nmol/l Ca^{2+} (35 min after c_7); a' , after 35 min stay of the retina in physiological saline (35 min after b').

The additional lowering of $[Na^+]_{ex}$ causes a strong diminution of h_{max} in the average from 51.5% ($\pm 2.7\%$) to 10.5% ($\pm 2.6\%$). The decay time t_2 is extremely prolonged and therefore not measurable during the recording period of 1 s. t_{max} does not change much; the latency period, however, is greatly prolonged (see Fig. 14). Although the excitability of the retina is strongly diminished the retina is not injured irreversibly. In the after-period in reference saline of the same high osmolarity h_{max} recovers again to 69% ($\pm 7\%$) after 20 min.

In the isolated retina of *Eupagurus* the raising of external $[Ca^{2+}]$ concomitant with corresponding lowering of the external $[Na^+]$ causes a stronger diminution of the ERG than that observed here at the *Astacus* retina by reduction of calcium in normal sodium concentration. This set of *Astacus* experiments with a combination of high calcium and low sodium shows a similar reduction of the ERG height.

The influence of decreased external calcium (10 mmol/l \rightarrow 1 nmol/l) and additional lowering of external sodium (208 \rightarrow 20.8 mmol/l)

The results are shown in Fig. 16 and Table IV.

The additional lowering of $[Na^+]_{ex}$ causes several changes of the ERG shape. The height of the ERG is reduced. The first record after the switch to a new saline shows two maxima which are followed by a slower rise of the potential height which is still continuing in the recording time (C ; after 5 min). The first maximum has a faster rise (t_{max} is about 40 ms) and decay than the second one (t_{max} is about 65 ms).

With prolonged stay in low sodium, low calcium saline, the first maximum becomes gradually smaller from 60% to about 40%. The second maximum is gradually reduced and finally disappears. Also time dependent the minimum after the first maximum becomes deeper and deeper.

When sodium is raised again the ERG shows a monophasic course with only one maximum and a very slow decay as in the preceding test period with the same saline (b' and b in Fig. 16).

The reversibility in the after-period in the reference saline is again quite good; h_{max} reaches 84.3% ($\pm 4\%$) after 35 min and the whole time course of the ERG is again similar to that of the pre-period in the reference saline.

Discussion

Raising external calcium changes the ERG in a direction which resembles light adaptation; lowering external calcium correspondingly causes changes of the ERG like dark adaptation in respect to height and decay of the ERG. It is tempting to assume that both, changes in external calcium on the one hand and adaptation on the other hand act on the same mechanism.

Our results can be interpreted in terms of the calcium-adaptation-hypothesis (Lisman and Brown [10]) in the following way: In low external $[Ca^{2+}]$ the light-induced raise in intracellular Ca^{2+} -concentration is diminished; in high $[Ca^{2+}]_{ex}$ the light induced raise is further increased.

A question of interest is, whether the external Ca^{2+} -concentration influences the internal Ca^{2+} -concentration during the dark period or during the light response.

To answer this question experiments were performed

- in a state of weak light adaptation (regular stimulus interval: 5 min) and
- in a state of practically complete dark adaptation (dark period: 60 min).

In external calcium concentrations between 10 and 50 mmol/l the dark adapted response is much greater and its decrease much slower than the weakly light adapted response (Fig. 8). However, in external calcium-concentrations of 150 mmol/l the dark adapted response shows no significant deviation from the weakly light adapted one (Fig. 9). These findings suggest that the increase in intracellular $[Ca^{2+}]$ with an external calcium-concentration of 10–100 mmol/l mainly occurs during the light-response, whereas in external calcium-concentrations > 100 mmol/l the internal Ca^{2+} -concentration increases already during the dark period. This increase in the dark dominates the light-induced change in intracellular $[Ca^{2+}]$.

For the same reason the sensitivity of the dark adapted *Limulus* ventral-nerve photoreceptor is not changed by varying $[Ca^{2+}]_{ex}$ between 1 nmol/l and 100 mmol/l (Stieve and Bruns (1979), unpublished).

The dependence of the rate of diminution in sensitivity on the Ca^{2+} -buffer (EDTA)-concentration suggests the existence of calcium stores which can be exhausted only very slowly by lack of calcium in the superfusate. It can be greatly enhanced by increasing the calcium-binding-capacity of the superfusate. According to investigations by Schröder, Frings, and Stieve [23], using the Laser Microprobe Mass Analyzer (Heinen *et al.* [24]) the distal shielding pigment in the retinula cell probably acts as calcium store.

It is conspicuous that the crayfish which shows this outstanding tolerance to low external calcium concentration, compared with other arthropods tested, is a fresh-water animal. It is adapted to tolerate Ca^{2+} lack by internal calcium stores.

In experiments with lowered external calcium in *Limulus* photoreceptor cells an antagonism between external calcium- and sodium-ions is observed (Stieve and Bruns [25]). In the *Astacus* retina in our experiments no antagonism of the action of external calcium and sodium on the height of the light response is observed but an antagonism on the dark adaptation. Lowering external calcium increases the rate of dark adaptation (Stieve and Hanani [14], Stieve, Bruns, Pflaum, and Gaube [26]), whereas lowering external sodium decreases it (Wulff [7],

Fein and Charlton [17]). In experiments with a constant stimulus interval, this effect can lead to the observed antagonistic action on the decrease time t_2 of the ERG (Fig. 16) – a parameter very sensitive to the state of adaptation.

The effect of lowering external sodium in lowered external calcium to cause the ERG to become polyphasic needs discussion. Several authors described observations which are easily explained by the assumption that the receptor potential is composed of at least two overlapping components (Benolken and Russell [27], Wulff and Mueller [28], Clark and Duncan [29], Maaz [18]). These different components are indicated by different maxima of the receptor potential which can be made observable by adjusting the stimulus condition. In *Limulus* ventral nerve photoreceptors the relative size of the 2 components C_1 and C_2 which constitute together the transient of the receptor potential is strongly affected by the state of light adaptation; C_2 is more sensitive to light adaptation than C_1 (Maaz, Nagy, Stieve, and Klotz [30]).

Under normal conditions in the crayfish retina by extracellular as well as intracellular recordings different components can only be seen rarely. Naka and Kuwabara [31] described two components of the ERG of the crayfish, but it is not absolutely sure whether they are generated solely from the receptor cells. In the experiments described here, however, in a combination of low calcium and low sodium the ERG shows regularly two maxima which presumably correspond to the components C_1 and C_2 observed in photoreceptors of other animals. Lowering $[Ca^{2+}]_{ex}$ or $[Na^+]_{ex}$ both act strongly on the state of adaptation. Possibly the combination of the effect of the two create transiently conditions for a pronounced difference in sensitivity of the two components to make both visible. It seems conceivable, that both components C_1 and C_2 are enlarged by low external calcium whereas C_2 is preferably diminished in low external sodium.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 160).

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