The Influence of the Extracellular Concentration of Calcium, Magnesium and Sodium on the Repolarizing Phase of the Receptor Potential of the *Limulus* Ventral Nerve Photoreceptor

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1. Lowering the extracellular calcium concentration from 10 mmol/l to 1 nmol/l causes, besides reducing membrane potential (PMP) and peak amplitude (h_{max}) of the light response of the *Limulus* ventral nerve photoreceptor (see Stieve and Bruns [1]), a prolongation of the time course of the light response.

The retarded time course (characterized by latent-period t_{lat}, time-to-peak t_{max}, decrease time t_2 and decline quotient Q_{HN}) caused by low calcium concentration is not antagonized by either reducing the sodium concentration (from 0.5 to 0.05 mol/l) or increasing the magnesium concentration (from 5×10^{-5} to 5×10^{-2} mol/l) in contrast to the effects on the PMP and h_{max}.

2. This effect of lowering the calcium concentration on the time course of the ReP is distinctly different from that on membrane potential and transient of the ReP described before.

It is not characterized by a calcium/sodium binding competition but is probably more closely related to the bump-generating mechanism. It can be explained on the assumption that the time parameters of the ReP are primarily determined by the latency distribution of the underlying bumps which is expanded under low calcium conditions.

Introduction

This investigation deals with the effects of changes of the extracellular ion concentration of calcium-, magnesium-, and sodium-ions on the time course of the receptor potential (ReP) of the *Limulus* ventral nerve photoreceptor.

In a previous publication [1] we have described the changes of the pre-stimulus membrane potential (PMP) and the height of the transient (h_{max}) of the intracellular recorded receptor potential upon lowering the $[Ca^{2+}]_{ex}$ together with normal and lowered $[Na^+]_{ex}$, and tested the effects of differently lowered $[Ca^{2+}]_{ex}$ as related to those of different $[Mg^{2+}]_{ex}$. The results were compared with those obtained by other authors in various arthropod species [2–9]. Based on experiments with *Limulus* (lateral eye) and *Astacus* [10–12] we developed the working hypothesis that calcium ions bound to the external membrane surface control the opening of light channels. Lowering of the $[Ca^{2+}]_{ex}$ decreases dark potential and light response height, it increases the dark conductance and reduces the light-induced conductance increase by opening of the light-activated ion channels.

We found that the effect of low calcium concentration (decreased to up to 10^{-9} mol/l) consists in a depolarization of the membrane potential and a decrease of the maximum of the light-evoked receptor potential [1]. This effect can be partially reversed by additionally lowering the sodium concentration. Concerning this action on the light response, calcium can be replaced by magnesium, though with a weaker effect.

In this study we want to check the action of calcium ions on the time course of the receptor potential, especially the repolarizing phase.

Methods and Procedure

Limulus ventral nerve photoreceptors were investigated in a standard way (for details see [1]).

White light from a xenon lamp (intensity ca. 90000 lx, corresponding to 1.5×10^{17} 550 nm photons $cm^{-2} s^{-1}$ was used; the light stimulus was of response height saturating intensity. The light responses (receptor potentials) were measured intracellularly by a microelectrode filled with 0.5 M KCl solution. The experiments lasted 3 to 4 h following

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successful impalement of the cell. The temperature of experiments was 15 °C. The preparations were kept in the dark except for 20 ms light flashes (evoking saturated response heights) applied every minute. Every fifth light response was recorded on tape. Each experiment consisted of up to five periods (a, b, c, d, e); in the first and last period the preparation was superfused with physiological saline. Generally in the b-period both the $[Na^+]_{ex}$ and the $[Ca^{2+}]_{ex}$ were decreased, while in the c-period the $[Na^+]_{ex}$ was again normal, but the $[Ca^{2+}]_{ex}$ was still low. In the d-period the same saline as in the b-period was used (and in the e-period the same as in the a-period). According to this scheme six groups of experiments with varied concentrations of calcium-, magnesium-, and sodium-ions were made. The duration of the periods was between 15 and 120 min, mostly 30 min, with the aim of reaching a steady state of the effects caused by the different salines (for composition of salines see Table I).

Evaluation

The receptor potentials were parallely recorded by a slow paper writer (Varian, speed 10 mm/min) and on oscilloscope, photographed, and stored on tape (Ampex) in periods of 5 s for evaluation by computer. The time resolution of the tape recordings was 1 ms, the accuracy of the voltage measurements 1 mV. The following parameters were determined by computer:

PMP [mV] the prestimulus membrane potential (difference between zero line (before penetration of the cell) and base line (intracellular recording) immediately before the light stimulus). The PMP was corrected for drift by taking the mean of initial and final zero as reference zero line. The PMP was determined as 100 ms average voltage before stimulus onset.

t_{lat} [msec] The latent period (time from stimulus onset until first measurable increase of the potential), determined by the first value exceeding the 3-fold value of the noise level.

t_{max} [msec] The time-to-peak, counted from stimulus onset.

h_{max} [mV] The peak amplitude of the response.

t_2 (msec) The decrease-time (the time between the measurement of h_{max} and $h_{max/2}$).

Q_{HN} The decline-quotient (the quotient h_N/h_{max}), which is a measure for the decline of the response; HN, the after-potential, is the response amplitude 500 ms after t_{max}; it was recorded as average voltage from 485 to 515 ms after measurement of t_{max}. The changes of the decline-quotient and of t_2 are in the same direction. (The Q_{HN} characterizes the decline even if the end of t_2 exceeds the total measuring interval of 5 s. In physiological saline at 15 °C the Q_{HN} is close to 0.5 for saturated responses; it rises to maximally 1 when there is no decline within 500 ms after the amplitude peak).

These values were normalized to a reference value of the same parameter obtained while the ventral nerve was superfused with physiological saline, except for the values of the decline-quotient Q_{HN} which were plotted as absolute values.

Results

Group A

Effects of 50 µmol/l Ca^{2+}, 5 mmol/l Mg^{2+} and low (38.4 mmol/l) or normal (542.8 mmol/l) $[Na^+]_{ex}$.

Experiments JB 146–148; Figs. 1, 2; Tables II, VII (lines 4, 5).

The changes of the RePs on lowering the $[Ca^{2+}]_{ex}$ to 50 µmol/l together with normal and low $[Na^+]_{ex}$ are shown in Fig. 1. In the low calcium, low sodium saline (b-period) the response is reduced in size, and all time parameters are prolonged. In the low calcium, normal sodium saline (c-period) the ReP becomes smaller. Except for t_{max}, which becomes slightly shorter, the time parameters are still more prolonged, *i.e.* the decline of the ReP is much slowed down. Lowering the sodium concentration again (d-period) causes a slight recovery of h_{max}. The decline (t_2) is not changed as compared to the c-period.

In the after-period in physiological saline t_{lat} and t_{max} approach their reference values, while t_2 does not recover completely.

Table I. Composition of salines used in six groups (A to F) of experiments in the ventral nerve photoreceptor of *Limulus*. The first saline (I) in each group is the physiological saline (in Group C and D also the second saline, with different buffer). Unless otherwise specified the ratio $[Na^+]_{ex}/[Ca^{2+}]_{ex}^{1/2}$ was kept constant as compared to physiological saline when both concentrations were lowered simultaneously. EGTA: Ethylene-glycol-bis (2-amino-ethyl ether) N,N′-tetra acetic acid. EDTA: Ethylene-diamine-tetra acetic acid. HEPES: N-2-hydroxy-ethyl piperazine-N′-ethyne sulfonic acid.

		Na^+ [mmol/l]	K^+ [mmol/l]	Ca^{2+} [mmol/l]	Mg^{2+} [mmol/l]	HEPES [mmol/l]	TRIS [mmol/l]	Cholin [mmol/l]	EGTA [mmol/l]	EDTA [mmol/l]	Cl^- [mmol/l]	SO_4^{2-} [mmol/l]	HCO_3^- [mmol/l]
A	I	542.8	10.0	10.0	5.0	10.0	–	–	–	–	577.8	–	–
	II	38.4	10.0	$5 \cdot 10^{-2}$	5.0	10.0	–	519.4	–	–	572.9	–	–
	III	542.8	10.0	$5 \cdot 10^{-2}$	5.0	10.0	–	14.9	–	–	572.8	–	–
B	I	542.8	10.0	10.0	5.0	10.0	–	–	2.0	–	537.4	–	–
	II	5.4	10.0	10^{-3}	5.0	10.0	–	552.6	2.0	–	569.6	–	–
	III	542.8	10.0	10^{-3}	5.0	10.0	–	15.2	2.0	–	569.6	–	–
C	I	491.0	10.0	10.0	55.0	–	–	–	–	–	626.0	–	5.0
	II	542.8	10.0	10.0	5.0	–	10.0	–	–	–	592.8	–	–
	III	38.4	10.0	$5 \cdot 10^{-2}$	$5 \cdot 10^{-2}$	–	10.0	526.9	–	–	585.3	–	–
	IV	542.8	10.0	$5 \cdot 10^{-2}$	$5 \cdot 10^{-2}$	–	10.0	22.4	–	–	585.3	–	–
D	I	488.3	10.0	10.0	55.0	–	–	–	–	–	566.0	30.0	2.3
	II	483.3	10.0	10.0	55.0	10.0	–	–	–	–	563.3	30.0	–
	III	48.3	10.0	$<10^{-6}$	$<10^{-4}$	10.0	–	517.5	–	1.0	575.8	–	–
	IV	483.3	10.0	$<10^{-6}$	$<10^{-4}$	10.0	–	82.0	–	1.0	575.3	–	–
E	I	468.3	10.0	10.0	55.0	10.0	–	–	–	–	608.3	–	–
	II	415.3	10.0	10^{-6}	100.0	10.0	–	–	1.0	–	625.3	–	–
	III	570.3	10.0	10^{-6}	$5 \cdot 10^{-2}$	10.0	–	–	1.0	–	580.3	–	–
F	I	488.3	10.0	10.0	55.0	–	–	–	–	–	566.0	30.0	2.3
	II	555.8	10.0	10.0	$5 \cdot 10^{-2}$	–	–	–	–	–	583.5	–	2.3

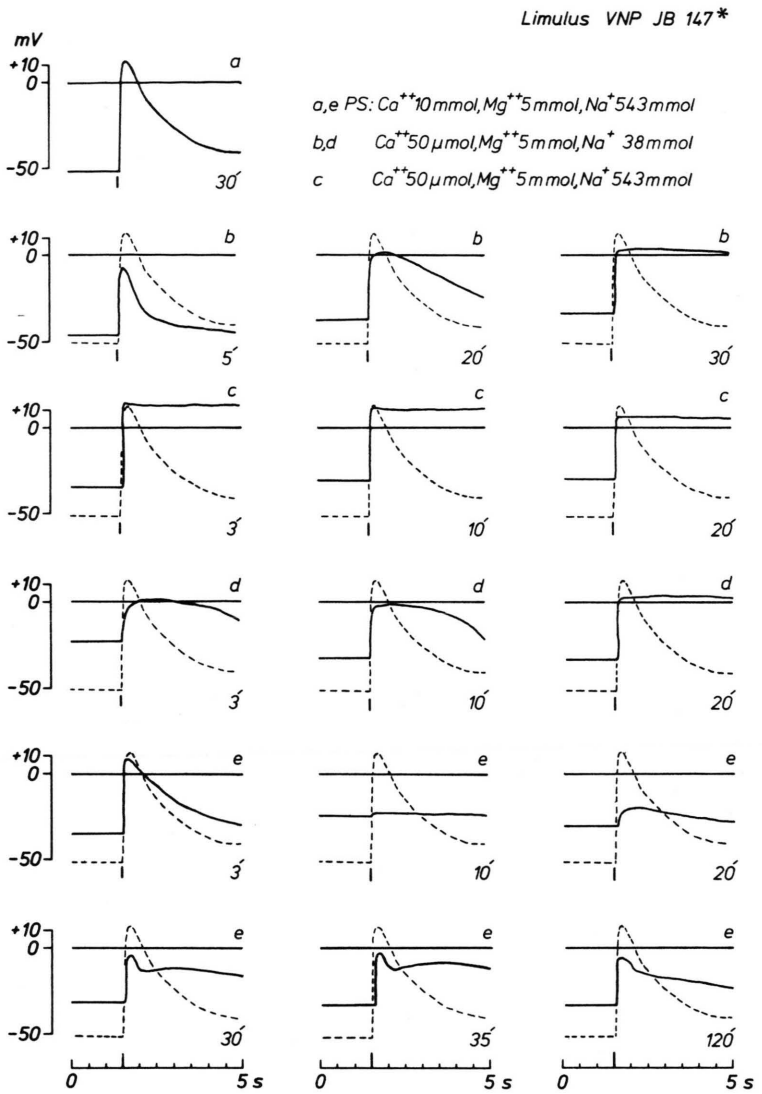

Fig. 1. Receptor potentials of *Limulus* ventral nerve photoreceptor recorded in low calcium (50 µmol/l) and low or normal sodium saline. 20 ms stimuli, white light, intensity corresponding to ca. 1.5×10^{17} 550 nm photons $cm^{-2}\,s^{-1}$. For salines see Table I (AI–AIII). a, e: physiological saline (AI), b, d: low calcium (50 µmol/l) low sodium (38.4 mmol/l), (AII), c: low calcium (50 µmol/l) normal sodium (542.8 mmol/l), (AIII). The reference potential recorded after 30 min in physiological saline (a) is drawn in broken line in each plot for comparison. The time of recording is indicated below the plots. The preparation was stimulated every minute, every 5th response was plotted. Temperature 15 °C. Experiment JB 147, Group A.

Fig. 2 shows the time-course of the changes of the PMP and h_{max}. Both parameters recover to a certain extent in the final period in physiological saline, after a transient "anesthetized" interval especially obvious for h_{max} immediately after the change to physiological saline, which was also observed in some of the other experiments, though less marked.

Group B

Effects of 1 µmol/l Ca^{2+}, 5 mmol/l Mg^{2+} and low (5.4 mmol/l) or normal (543.8 mmol/l) $[Na]_{ex}$.

Experiments JB 142a – 145) Table VII (lines 6, 7).

The results of this group are not shown in a separate table or figure, but only in the collective table, because they are basically similar to those of Group A. The experimental difference is only that the $[Ca^{2+}]_{ex}$ is still more reduced.

The only apparent difference to the results of Group A consists in the change of the latent period. While t_{lat} is more increased in low calcium normal sodium saline than in low calcium low sodium in Group A, the opposite applies to Group B where the $[Ca^{2+}]_{ex}$ is more reduced (to 1 µmol/l). The

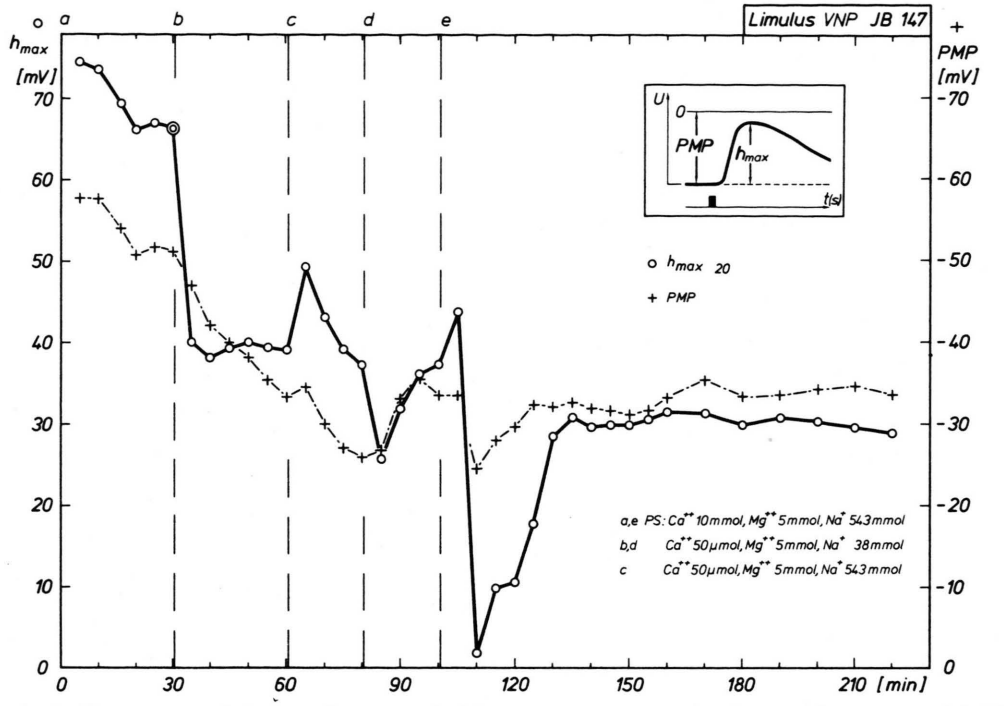

Fig. 2. Time course of change of response height ($h_{max\,20}$) and pre-stimulus membrane potential (PMP) during stay in physiological saline (I), low calcium and low sodium saline (II) and low calcium and normal sodium saline (III). Same experiment as in Fig. 1, Group A.

decrease-time t_2 is less prolonged under sodium and calcium deficiency than in low calcium normal sodium saline.

Group C

Effects of 50 µmol/l Ca^{2+}, 50 µmol/l Mg^{2+} and low (38.4 mmol/l) or normal (542 mmol/l) $[Na]_{ex}$.

(Experiments JB 152–155), Tables III, VII (lines 8, 9).

In this group the $[Mg^{2+}]_{ex}$ was lower than in those described before.

In the low calcium, low magnesium, low sodium saline (b-period) the ReP becomes longer, but only slightly reduced in size. Under normal sodium conditions (calcium and magnesium still low, c-period) the response becomes much smaller, and the PMP becomes positive. The decline remains prolonged. Back in the same saline as in the b-period (again low sodium) the response does not recover, but remains at a low level even in the after-period.

The values of t_{lat} are shorter under calcium and magnesium deficiency when sodium is present (c-period), than when it is also reduced (b- and d-period). The same applies to t_{max}. The decline phase of the response, characterized by t_2 and the Q_{HN}, is increasingly prolonged throughout the experiment and not reversed within 30 min in physiological saline.

Group D

Effects of very low extracellular concentration of divalent cations (< 1 nmol/l Ca^{2+} and 100 nmol/l Mg^{2+}) and low (48.3 mmol/l) or normal (483.3 mmol/l) $[Na^+]_{ex}$. The ratio $[Na^+]/[Ca^{2+}]^{1/2}$ was increased. Experiments JB 149–151; Fig. 3; Tables IV and VII (lines, 10, 11).

We wanted to test the effect of an extremely low extracellular divalent cation concentration without damaging the preparation too much. For this reason the test periods b, c, and d were kept 10 to 15 min. (The time-course of one experiment, JB 151, is shown in Stieve and Bruns [1], Fig. 1.) As Fig. 3 shows, the changes of the RePs were fast and almost stationary, and recovery in physiological saline was good.

Table II, Group A. Effect of 50 μmol/l Ca^{2+} and low or normal $[Na^+]$ on the ReP of the *Limulus* ventral nerve photoreceptor. Intracellular measurement of pre-stimulus membrane potential PMP, saturated response height h_{max}, latent period t_{lat}, time-to-peak t_{max}, decrease-time t_2 and decline-quotient Q_{HN}. Letters a to d indicate successive periods in different salines. For composition of salines see Table I. AI: physiological saline, HEPES buffer; AII: low calcium (50 μmol/l), low sodium (38.4 mmol/l); AIII: low calcium (50 μmol/l), normal sodium (542.8 mmol/l). Asterixes mean that the respective time was longer than the interval of measurement. Negative signs in brackets show the voltage direction of the PMP as compared to outside. 20 ms white light stimuli of intensity corresponding to ca. 1.5×10^{17} 550 nm photons $cm^{-2}\ s^{-1}$. Experiments JB 146–148, $n = 3$, unless indicated in brackets.

	PMP	h_{max}	t_{lat}	t_{max}	t_2	Q_{HN}
a 30 min A I	−38± 6.5 mV	54± 5.5 mV	33± 4.1 ms	125± 26 ms	783±267 ms	0.58±0.11
b_1 20 min A II	(−) 46± 5.1%	55± 5.1%	141± 2.8%	928±658%	133± 65%	0.81±0.12
b_2 30 min A II	(−) 49± 8.7%	47± 6.9%	151± 14%	545± 52%	556% (1)	0.98±0.003
c_1 10 min A III	(−) 44± 7.9%	54± 6.3%	159± 44%	327±127%	*	0.96±0.01
c_2 20 min A III	(−) 29±13%	37±12%	233±112%	568±283%	342± 86% (2)	0.97±0.01
d_1 10 min A II	(−) 50±10%	24±14%	169± 66%	901±598% (2)	224% (1)	0.97±0.01 (2)
d_2 20 min A II	(−) 58± 8.7%	40±17% (2)	180± 82% (2)	1476±860% (2)	*	0.98±0.02 (2)
e_1 10 min A I	(−) 37±10%	4± 2.6%	220± 28% (2)	155± 45% (2)	57± 37% (2)	0.52±0.36 (2)
e_2 30 min A I	(−) 32±16%	14±14%	175% (1)	151% (1)	*	0.65 (1)
e_3 60 min A I	(−) 39±27%	24±24%	103% (1)	208% (1)	208% (1)	0.68 (1)
e_4 120 min A I	(−) 67% (1)	43.2% (1)	105% (1)	80% (1)	173% (1)	0.73 (1)

Table III, Group C. Effect of 50 μmol/l Ca^{2+}, 50 μmol/l Mg^{2+} and low or normal $[Na^+]$ on the ReP of the *Limulus* ventral nerve photoreceptor. Further details as in Table II. For composition of salines see Table I. Letters a to e indicate successive periods in different salines. CI: physiological saline with 10 mmol/l Ca^{2+}, 491 mmol/l Na^+, 55 mmol/l Mg^{2+}, buffer HCO_3^-, CII: physiological saline with 10 mmol/l Ca^{2+}, 5 mmol/l Mg^{2+}, 528.8 mmol/l Na^+, buffer Tris, CIII: low calcium (50 μmol/l) low magnesium (50 μmol/l), low sodium (38.4 mmol/l); CIV: low calcium (50 μmol/l), low magnesium (50 μmol/l), normal sodium (542.8 mmol/l). Experiments JB 152–155, $n = 4$.

	PMP	h_{max}	t_{lat}	t_{max}	t_2	Q_{HN}
a 15 min C I	−37± 3.5 mV	54± 3.0 mV	30± 7.3 ms	159± 59 ms	638±284 ms	0.46±0.11
a′ 15 min C II	(−) 115± 7.4%	106± 7.8%	129±16%	127± 30%	336±224%	0.54±0.07
b_1 20 min C III	(−) 62±12%	53± 9.8%	187±29%	640±335%	997±436%	0.95±0.01
b_2 30 min C III	(−) 70±35% (2)	56±17% (2)	215±35%	1036±585%	1613% (3)	0.97±0.02 (2)
c_1 10 min C IV	(+) 5± 2.2%	18± 8.3%	119± 8.0% (3)	187± 48%	1600± 98% (2)	0.87±0.03 (3)
c_2 15 min C IV	(+) 10± 0.9% (2)	17±13% (2)	134±18% (2)	376±257%	1953% (1)	0.87±0.01 (2)
d_1 10 min C III	(−) 37±12%	10± 4.9%	364±69% (3)	577±207% (3)	444±218% (3)	0.69±0.11 (3)
d_2 20 min C III	(−) 45±22% (2)	12±12% (2)	467% (1)	1007% (1)	1132% (1)	0.83
e_1' 10 min C II	(−) 4± 5.8%	3± 3.0%	629% (1)	917% (1)	1189% (1)	0.73 (1)
e_2' 30 min C II	(−) 12±13% (3)	9± 8.9% (3)	675% (1)	1160% (1)	1061% (1)	0.81 (1)
e_1 30 min C I	(−) 6±10% (3)	6± 5.7% (3)	725% (1)	1057% (1)	821% (1)	0.73 (1)

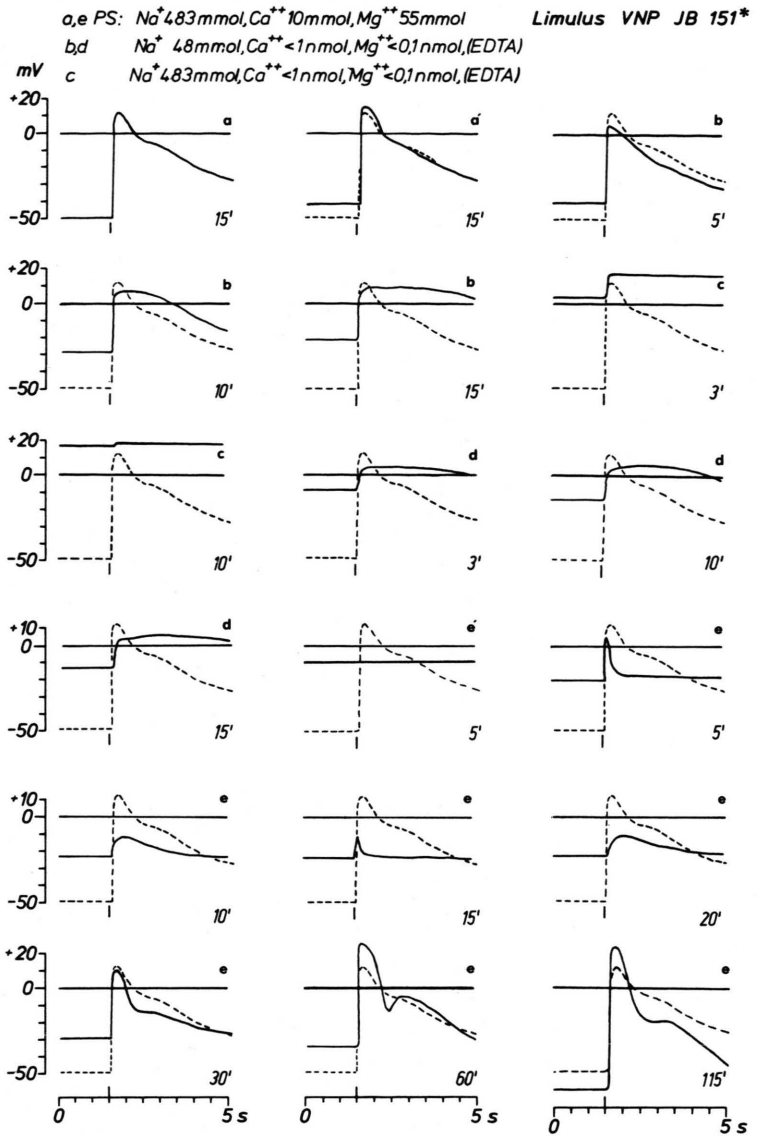

Fig. 3. Receptor potentials recorded in low calcium (1 nmol/l) low magnesium (100 nmol/l) and low or normal sodium saline. Stimulus conditions as in Fig. 1; for salines see Table I (DI–DIV). a, e: physiological saline with HCO_3 (DI), a′, e′: physiological saline with HEPES (DII), b, d: low calcium (1 nmol/l), low magnesium (100 nmol/l), low sodium (48.3 mmol/l) saline with EDTA (DIII), c: low calcium (1 nmol/l), low magnesium (100 nmol/l), normal sodium (483.3 mmol/l) saline with EDTA (DIV). Experiment JB 151, Group D.

In the b-period (low calcium, magnesium and sodium) the time-parameters where slightly prolonged. In the c-period (low calcium, normal sodium) the response became very small, with very long values of t_{lat} and t_{max} (over 1000%), but a decrease-time t_2 near the reference value. In the d-period (same saline as b-period) the response was partially restored, but with more prolonged values of t_{lat} and t_{max} than in the b-period. As shown by the high value of the decline-quotient Q_{HN} in the d-period the decrease-time was very long. In the after-period the response recovered well within an hour.

Group E

Effects of low $[Ca^{2+}]_{ex}$ (1 nmol/l) and high $[Mg^{2+}]_{ex}$ (100 mmol/l) and low $[Ca^{2+}]_{ex}$ and low $[Mg^{2+}]_{ex}$ (50 µmol/l).

Experiments JB 135, 136, 138, 139; Figs. 4, 5; Tables V, VII (lines 12, 13).

Table IV, Group D. Effect of 1 nmol/l Ca^{2+}, < 100 nmol Mg^{2+} and low or normal $[Na^+]$ on the ReP of the *Limulus* ventral nerve photoreceptor. Further details as in Table II. For composition of salines see Table I. Letters a to e indicate successive periods in different salines. DI: physiological saline, buffer HCO_3, DII: physiological saline, buffer HEPES, DIII: low calcium (1 nmol/l), low magnesium (< 100 nmol/l), low sodium (48.3 mmol/l), buffer EDTA, DIV: low calcium (1 nmol/l), low magnesium (< 100 nmol/l). Experiments JB 149–151, $n = 3$.

		PMP	h_{max}	t_{lat}	t_{max}	t_2	Q_{HN}
a	15 min D I	−50± 3.0 mV	63 ± 1.5 mV	36± 4.6 ms	139± 11 ms	1169±616 ms	0.57±0.15
a′	15 min D II	(−) 94± 5.8%	96 ± 2.4%	113± 7.9%	109± 5.9%	78± 12%	0.52±0.13
b	10 min D III	(−) 52± 9.3%	50 ± 9.1%	153± 6.4%	123± 23%	113± 15%	0.66±0.17
c	10 min D IV	(+) 23± 9.6% (2)	3.8± 1.8% (2)	1146±860% (2)	1242±1019% (2)	97% (1)	0.7
d	10 min D III	(−) 20± 7.2% (2)	20 ±11% (2)	357±127% (2)	1443± 533% (2)	*	0.95
e′	5 min D II	(−) 18±11%	3.8± 3.8%	27% (1)	103% (1)	54,2% (1)	0.25
e	15 min D I	(−) 22±25% (2)	11 ± 8.9% (2)	260±115% (2)	193± 117% (2)	32± 29% (2)	0.38±0.23 (2)
e_2	30 min D I	(−) 34±22% (2)	32 ±30% (2)	60± 54% (2)	1378±1240% (2)	19% (1)	0.45 (17
e_3	60 min D I	(−) 69% (1)	95% (1)	89% (1)	99% (1)	28% (1)	0.73 (1)

Table V, Group E. Magnesium as substitute for calcium in *Limulus* ventral nerve photoreceptor. Further details as in Table II. For composition of salines see Table I. Letters a to e indicate successive periods in different salines. EI: physiological saline, buffer HEPES, EII: low calcium (1 nmol/l), high magnesium (100 mmol/l), buffer EDTA, EIII: low calcium (1 nmol/l), low magnesium (50 µmol/l), buffer EGTA. Experiments JB 135–138, 139, $n = 4$.

		PMP	h_{max}	t_{lat}	t_{max}	t_2	Q_{HN}
a	30 min E I	− 43 ±11 mV	61±12 mV	43± 3.6 ms	130± 4.9 ms	1238±344 ms (3)	0.78±0.08
b_1	20 min E II	(−) 72 ±18%	75±17%	194± 24%	1460±469%	423% (1)	0.91±0.07 (3)
b_2	30 min E II	(−) 87 ±12% (3)	74±17% (3)	203± 53% (3)	542±366% (3)	556% (1)	1.02±0.15 (3)
c_1	10 min E III	(−) 53 ±30%	53±20%	161± 31%	1199±364%	* (4)	0.95±0.02
c_2	20 min E III	(+) 0.04±10% (3)	20± 7.6% (3)	138± 32% (3)	1069±469% (3)	* (3)	0.93±0.02 (3)
d_1	15 min E II	(−) 58 ±15%	38±15%	1487±1097%	1881±397%	* (4)	0.97±0.02 (2)
d_2	30 min E II	(−) 79 ± 0.5% (2)	52±13% (2)	419± 199% (2)	2013±440% (2)	* (2)	0.98 (1)
e_1	30 min E I	(−) 72 ±19%	12±10%	1542±1355% (3)	838±380% (3)	81±34% (2)	0.86±0.14 (3)
e_2	60 min E I	(−) 68 ±30% (3)	18±15% (3)	1061± 876% (3)	381±272% (3)	85±67% (2)	0.51±0.25 (3)
e_3	90 min E I	(−) 78 ±14% (2)	52± 4.9% (2)	126± 20% (2)	97± 9.4% (2)	71% (1)	0.55±0.07 (2)
e_4	120 min E I	(−) 63 ± 5.9% (2)	58± 1.7% (2)	128± 18% (2)	131± 26% (2)	12% (1)	0.47±0.17 (2)
e_5	150 – 170 min E I	(−) 61 ± 1.9% (2)	63± 2.5% (2)	107± 5.0% (2)	117± 73% (3)	18% (1)	0.48±0.10 (2)

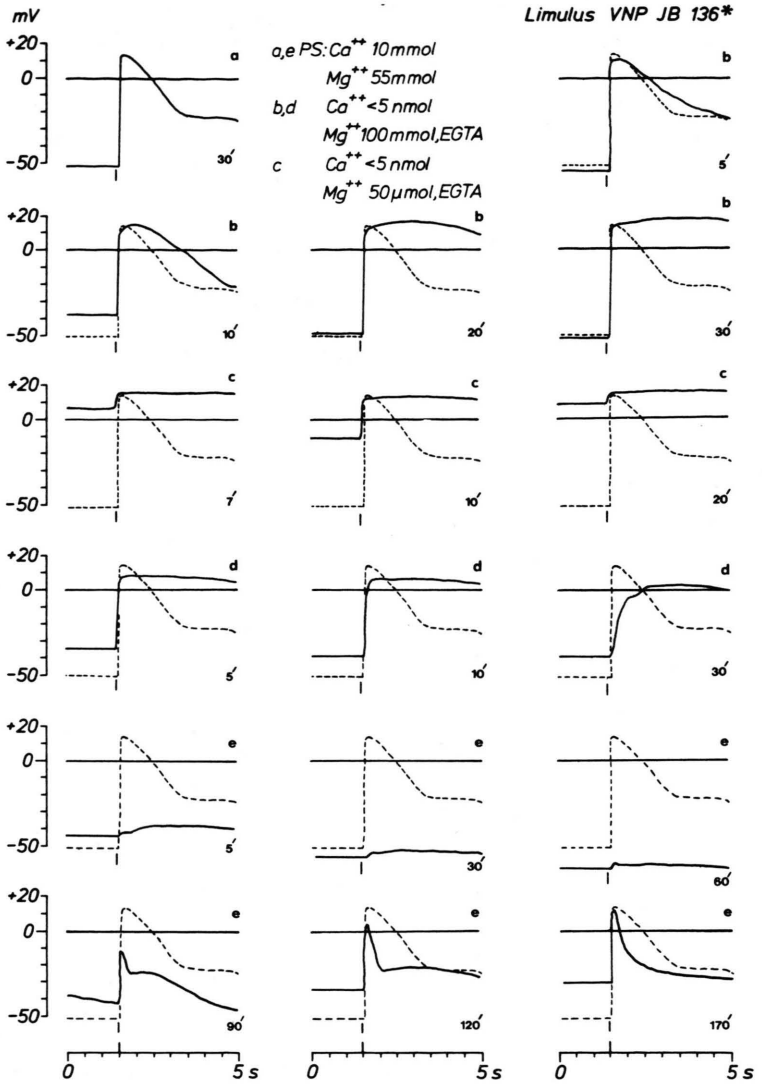

Fig. 4. Receptor potentials recorded in a saline with magnesium as substitute for calcium. Stimulus conditions as in Fig. 1; for salines see Table I (EI–EIII). a, e: physiological saline, b, d: low calcium (1 nmol/l), high magnesium (100 mmol/l) saline with EGTA (EII), c: low calcium (1 nmol/l), low magnesium (50 µmol/l), saline with EGTA (EIII). Experiment JB 136, Group E.

The shape and size of the RePs is very much changed under the conditions of calcium and magnesium deficiency.

The RePs recorded in low calcium, high magnesium saline (b-period) are slightly smaller, but all time parameters were very much prolonged (decline-quotient Q_{HN} ca. 1.0). In the low calcium, low magnesium saline (c-period) the decline phase remains prolonged, and the RePs become very small in size.

Back in the low calcium, high magnesium saline (d-period) h_{max} recovers well, but the time parameters become even more prolonged (Fig. 4).

Upon return to physiological saline t_{lat} recovers slowly to its reference value.

t_{max} is reduced to a value around its reference value after 90 min but slightly increases again towards the end of the after-period. The decline phase is very much shortened, to shorter values than in the pre-period, in the after-period (t_2 ca. 20%, Q_{HN} below 0.5).

Directly after return to physiological saline the response (h_{max}) is markedly anesthetized (Fig. 5) for almst one hour, while the pre-stimulus membrane potential recovers well.

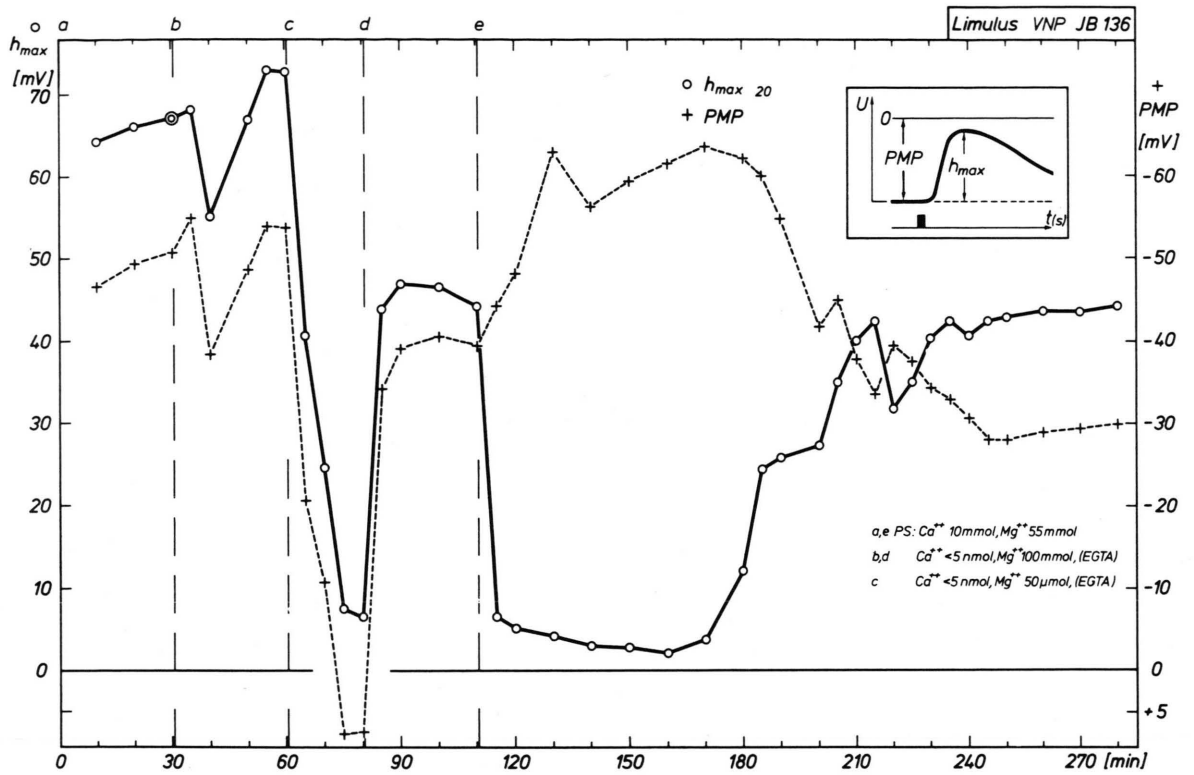

Fig. 5. Time course of change of response height ($h_{max\,20}$) and pre-stimulus membrane potential (PMP) during stay in physiological saline (I), low calcium (1 nmol/l), high magnesium (100 nmol/l) saline (II), and low calcium (1 nmol/l), low magnesium (50 µmol/l) saline (III). Same experiment as in Fig. 6. Experiment JB 136, Group E.

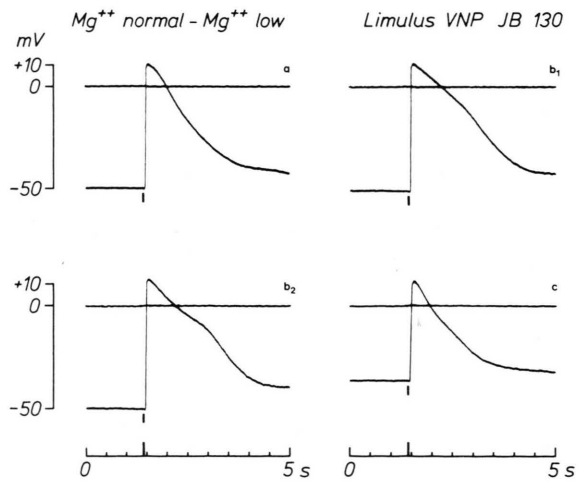

Fig. 6. Receptor potentials recorded in a low magnesium saline. Stimulus conditions as in Fig. 1; for salines see Table I (FI, II). a: 6 min in physiological saline (FI), b_1: 26 min in low magnesium (50 µmol/l) saline (FII), b_2: 56 min again in FI, c: 38 min again in FI. Experiment JB 130, Group F.

Group F

Long-term effects of low $[Mg^{2+}]_{ex}$ and normal $[Ca^{2+}]_{ex}$ and $[Na^{+}]_{ex}$.

Experiments JB 128–130; Fig. 6, Tables VI, VII (line 3).

Preparations were exposed to low $[Mg^{2+}]_{ex}$ (50 µmol/l) for ca. 2 h. The calcium and sodium concentration of the saline was normal. The changes are small. The PMP and h_{max} are only slightly reduced in the low magnesium period. They do not recover in the after-period but remain reduced. The effect of the low magnesium saline on the time course of the ReP consists mainly in a small prolongation (to ca. 130%) of the decrease-time. In the after-period t_2 is significantly reduced to ca. 50%. The time-to-peak is slightly shortened (to 80%) in the b-period and increases to ca. 160% in the after-period. The latent-period was not measured in these experiments.

Table VI, Group F. Effect of low magnesium (50 µmol/l) on the ReP of the *Limulus* ventral nerve photoreceptor. Intracellular measurement of pre-stimulus membrane potential PMP, saturated response height h_{max}, time-to-peak t_{max} and decrease-time t_2 (these values were measured by eye and referred to the average values between 26 and 38 min in physiological saline; first line). Letters a to c indicate successive periods in different salines (for composition see Table I). Further details as in Table II. FI: physiological saline, buffer HCO_3, FII: low magnesium (50 µmol/l). Experiments JB 128 – 130, $n = 3$; time of measurements within specified intervals.

		PMP	h_{max}	t_{max}	t_2
a	26 – 38 min F I	− 55± 4.8 mV	75±8.8 mV	140±30 ms	1349±135 ms
b_1	26 – 32 min F II	(−) 96± 4.2%	98±3.6%	88± 3.2%	121± 16%
b_2	56 – 58 min F II	(−) 88± 8.0%	98±7.3%	81± 3.2%	130± 23%
c	28 – 60 min F I	(−) 42±20%	73±5.6%	162±42%	47± 20%

Summary of the Results

The results of Group A to E are compiled in Table VII to allow a direct comparison of the changes brought about by the different ionic composition of the salines.

1. Effects of lowering the calcium concentration

When comparing the effect of increasingly lowered $[Ca^{2+}]_{ex}$ together with normal $[Na^+]_{ex}$ (Groups A, B, D; lines 4, 6, and 10) we find depolarized values for the membrane potential (PMP) and a decrease of the amplitude of the transient (h_{max}). The time parameters are prolonged; t_{lat} and t_{max} increasingly with decreasing $[Ca^{2+}]_{ex}$. The decrease-time t_2 is much prolonged, but not proportionally with the change of the $[Ca^{2+}]_{ex}$. Lines 5, 7, and 11 were obtained in the same experiments, with correspondingly lowered $[Ca^{2+}]_{ex}$ but with additionally lowered $[Na^+]_{ex}$ (to 10% in all cases). The additional lowering of the $[Na^+]_{ex}$ has the following effects: It counteracts the effect of low $[Ca^{2+}]_{ex}$ on the PMP and h_{max}, improving them in the direction of the reference value. It improves the latent-period slightly for some calcium concentrations, for others (line 7) not: here it is prolonged. It prolongs the time-to-peak is even more than the latent-period, but it has no significant effect on t_2 or the decline quotient Q_{HN}.

When both $[Ca^{2+}]_{ex}$ and $[Mg^{2+}]_{ex}$ are low (Group C) the additional lowering of the $[Na^+]_{ex}$ (line 9) leads to improvement of the PMP and h_{max}, but not of the time parameters. Both t_{lat} and t_{max} are somewhat more prolonged than in the low calcium, normal sodium saline. The decline of the response is not significantly changed. The effects on all parameters are quite similar to those just described for higher $[Mg^{2+}]_{ex}$: recovery of the PMP and h_{max}, which had been altered by low calcium alone, but no substantial changes of the time parameters.

2. Effects of altered magnesium concentration

When the $[Mg^{2+}]_{ex}$ is lowered, while the $[Ca^{2+}]_{ex}$ is also low (and the $[Na^+]_{ex}$ is normal) the effects compared to the reference value are these (lines 8 and 12, Group C and E): Pre-stimulus membrane potential PMP and transient of the response h_{max} are reduced. The latency is not significantly changed, time-to-peak and decrease-time are prolonged, t_2 very much. The decline-quotient is increased accordingly.

The effect of increased $[Mg^{2+}]_{ex}$, together with low $[Ca^{2+}]_{ex}$ and normal $[Na^+]_{ex}$ can be seen by comparing Group C (line 8) with Group A (line 4), and line 12 with line 13 (Group E). The effect of low calcium on membrane potential and response height is partly counteracted by increased $[Mg^{2+}]_{ex}$, but the latent-period and the time-to-peak are even more prolonged, and the decrease-time is not changed significantly.

The additional lowering of sodium improves the PMP and h_{max}, if the $[Mg^{2+}]_{ex}$ and the $[Ca^{2+}]_{ex}$ are low (line 11, Group D, line 9, Group C). There is no specific effect of lowering the $[Na^+]_{ex}$ on the time parameters.

Collective Table VII. Influence of different concentrations of Ca^{2+}, Mg^{2+} and Na^{+} on the ReP of the *Limulus* ventral nerve photoreceptor: Compilation of the results of Groups A to E. Values of pre-stimulus membrane potential (PMP), saturated response height (h_{max}), latent-period (t_{lat}), time-to-peak t_{max}, decrease-time (t_2) and decline-quotient (Q_{HN}) are average last values of the stay in the respective saline. The upper values of Groups A to D were recorded in the c-period (saline with normal sodium concentration, lines 4, 6, 8, 10). The lower values of Groups A to D (lines 5, 7, 9, 11) are averaged last values of the b and d periods recorded in low sodium saline. The upper value of Group E was recorded in the c-period with low magnesium (line 12), and the lower values of Group E were averaged from the b and d periods with high magnesium saline (line 13). The values were normalized for the single groups and the scale obtained by averaging all reference values for the respective parameter (line 1, PS). Line 2 shows low sodium and line 3 low magnesium values (other concentration unchanged) for comparison. The low sodium values of line 2 are in brackets because they were recorded in *Limulus* lateral eyes under identical conditions. For experimental details see legends of Table I to VI.

	Ca^{2+} [mmol/l]	Mg^{2+} [mmol/l]	Na^{+} [mmol/l]	n	PMP [mV]	h_{max} [mV]	t_{lat} [ms]	t_{max} [ms]	t_2 [ms]	Q_{HN}
1 PS	10	5	542.8	22	−44 ±2.7	60 ±3.0	35±2.2 (19)	146± 16	981± 141 (21)	0.59±0.05
2 low Na^{+}	10	5	1.4×10^{-2}	4	(−32 ±5.3)	(36 ± 3.2)	(39±3.2)	(121± 18)	(697± 242)	–
3 low Mg^{2+}	10	5×10^{-2}	550.0	3	−42 ±1.8	59 ± 2	–	128± 4.7	1187± 157	–
4 Group A	5×10^{-2}	5	542.8	3	−13 ±5.7	22 ± 7.2	82± 39	829± 413	3355± 844 (2)	0.97±0.01
5 Group A	5×10^{-2}	5	38.4	6	−22 ±2.7	22 ± 5.5	57± 9.8 (5)	1339± 520 (5)	5435 (1)	0.98±0.01
6 Group B	10^{-3}	5	542.8	5	−10 ±7.0	23 ±10	209±163 (4)	1813± 946 (4)	* (4)	0.98±0.01 (3)
7 Group B	10^{-3}	5	5.4	10	−21 ±3.6	23 ± 5.2	265±185 (9)	2180± 682 (9)	2315±1315 (4)	0.89±0.03 (6)
8 Group C	5×10^{-2}	5×10^{-2}	542.8	4	− 3.1±1.3	8.4± 4.3	46± 3.5 (3)	438± 244 (3)	24074±3767 (2)	0.86±0.01 (3)
9 Group C	5×10^{-2}	5×10^{-2}	38.4	8	−16 ±6.2	16 ± 5.6	97± 21 (6)	1248± 307 (6)	10860±3522 (3)	0.89±0.05 (6)
10 Group D	$<10^{-6}$	$<10^{-4}$	483.3	2	−10 ±4.2	2.3± 1.1	401±301	1813±1488	952 (1)	0.7 (1)
11 Group D	$<10^{-6}$	$<10^{-4}$	48.3	5	−12 ±2.1	18 ± 3.7	87± 21	1221± 453	549± 324 (2)	0.9 ±0.09 (4)
12 Group E	10^{-6}	5×10^{-2}	570.3	4	+ 0.1±3.2	10 ± 3.8	56± 11	1850± 564	*	0.92±0.02
13 Group E	10^{-6}	100	415.3	8	−30 ±4.8	29 ± 6.6	312±195	2256± 489	5454 (1)	0.99±0.07

Conclusions

The fact that both sodium reduction and magnesium increase have a restoring effect on the changes of membrane potential and voltage response height of the *Limulus* ventral nerve photoreceptor induced by lowering the calcium concentration of the external saline [1] has been explained on the basis of a sodium/calcium antagonism: Opening and closing of "light channels" in the visual cell membrane is controlled by negative binding sites for which calcium and sodium ions compete. The channels are closed when calcium is bound and open when sodium is bound. The effect of magnesium ions in this respect is similar to that of calcium ions, though weaker.

Our present results show that the action of calcium on the time parameters of the response is clearly different from that on membrane potential and response amplitude described above. The effect of calcium reduction on the time course of the response, which consists in a prolongation of all time parameters, is not counteracted by either sodium reduction or magnesium increase. The action of calcium on the duration of the response is not characterized by a calcium/sodium binding competition.

The calcium-dependent effect on the duration of the response (influencing latent-period, time-to-peak, and decrease-time in the same direction) is probably connected with the bump generating mechanism: Size and time distribution of the bumps, of which the ReP is composed, primarily determine its time course. We demonstrated [13] that the bump latency distribution is broadened and shifted to longer latencies when the calcium concentration is lowered. This could explain the slowing down of the response, especially of the decline, in low calcium saline.

The decline of the response is a sensitive measure for the state of adaptation of the photoreceptor. Light adaptation, as well as a rise in the $[Ca^{2+}]_{ex}$ sharpens the bump latency distribution and shifts it to shorter latencies [13, 14]. This makes plausible that also the changes in the time course of the ReP due to light adaptation are primarily based on changes in the bump latency distribution.

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