The Sensitivity Shift Due to Light Adaptation Depending on the Extracellular Calcium Ion Concentration in *Limulus* Ventral Nerve Photoreceptor

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Z. Naturforsch. **39 c,** 662–679 (1984); received February 23, 1984

Limulus Ventral Nerve Photoreceptor, Reproducible States of Light and Dark Adaptation, Intensity Dependence, Calcium Dependence of Light Adaptation, Sodium and Magnesium Ions

Receptor potentials of *Limulus* ventral photoreceptors were recorded in two defined states of moderate light- and considerable dark adaptation (LA, DA) by a repeated stimulus sequence consisting of a conditioning 2 s illumination (white light, response saturating intensity) followed by two 10 ms test flashes at fixed intervals evoking LA and DA responses (intensity varied from threshold to saturation of response amplitude). The half saturating intensity I_{50} was determined from response height *vs* log stimulus intensity curves for LA and DA, while the photoreceptor was superfused either by reference saline (physiological ion concentrations, including 10 mmol/l Ca^{2+}) or by test salines in which the $[Ca^{2+}]$ was varied between 40 µmol/l and 100 mmol/l. The sensitivity of the dark-adapted receptor does not significantly depend on the $[Ca^{2+}]_{ex}$, but the sensitivity shift due to LA (measured by I_{50}) is reduced when the $[Ca^{2+}]_{ex}$ is lowered, and augmented when the $[Ca^{2+}]_{ex}$ is increased. Additional reduction of the $[Na^{2+}]_{ex}$ from 463 mmol/l to 46 mmol/l or increase of the $[Mg^{2+}]_{ex}$ from 50 mmol/l to 100 mmol/l does not counteract the effect of lowered $[Ca^{2+}]_{ex}$ on LA. The results confirm the assumption that a transient increase of the intracellular $[Ca^{2+}]$ supplied from extracellular sources during the light response is the main cause for LA. This calcium effect on light adaptation is neither characterized by a calcium/sodium antagonism, nor mimicked by magnesium, in contrast to the calcium effect on the gating of the light-activated ion channels.

Introduction

In a previous publication [1] we described the basic feature of light and dark adaptation in *Limulus* ventral photoreceptors by using a repeated flash sequence evoking reproducible states of moderate light and considerable dark adaptation. These results were obtained with physiological saline as bathing solution and without background illumination to demonstrate the effect of a) the state of adaptation and b) the stimulus intensity on height and time parameters of the light response (receptor potential) of the photoreceptor under conditions close to normal.

The same experimental procedure was adopted for the experiments presented here which are intended to show how the response versus intensity characteristics of the *Limulus* photoreceptor in the state of light and dark adaptation are influenced by different calcium concentrations of the bathing saline. Calcium plays a decisive role in the generation of the light response and in light adaptation [2–6]. By varying the extracellular calcium concentration in the range from 40 µmol/l to 100 mmol/l and evaluating the effects separately for the different parameters of the light response we obtained more detailed information on the action of calcium in the photoreceptor in the different states of adaptation.

In some experiments we also varied the extracellular sodium concentration to check whether a calcium/sodium antagonism exists concerning any of the effects of changed extracellular calcium concentration on light and dark adaptation under the conditions studied. A calcium/sodium antagonism was described for the excitation mechanism (gating of the light activated ion channels) in *Limulus* photoreceptors by Stieve and Bruns, [7], and for the barnacle photoreceptor by H. M. Brown *et al.* [8].

Since magnesium can replace calcium, but with a weaker effect, in the calcium action on the gating of the light-activated ion channels, which is characterized by a calcium/sodium antagonism [7], we also applied a test saline with low calcium and raised

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0341-0382/84/0600-0662 $ 01.30/0

magnesium concentration to study whether magnesium can replace calcium also in its action on light adaptation.

Methods

Limulus ventral nerves were excised and mounted in a test vessel, where they were superfused with physiological saline (PS) or different test salines (for composition see Table I) at a flow rate of about 1 ml/min. We used two Varian xenon lamps (VIX 150) emitting white light, one for a conditioning, light adapting, illumination with a maximal intensity equivalent to ca. $1.8 \cdot 10^{16}$ (550 nm) photons $cm^{-2}\,s^{-1}$; and one for test flashes, with a maximal intensity equivalent to ca. 9×10^{16} (550 nm) photons $cm^{-2}\,s^{-1}$. The light intensity was varied by neutral density filters (Schott).

The light-evoked membrane voltage signal (receptor potential, ReP) of the ventral nerve photoreceptor was measured intracellularly by a microelectrode filled with 0.5 mol/l KCl against an indifferent silver/silver chloride electrode in the bathing saline. The experiments were carried out at 15 °C. The results were recorded by a slow paper writer (Hellige, speed 1 cm/min), photographed, stored on tape and evaluated either by hand or by computer.

For a closer description of the methods see [1], details of the evaluation are described in [9].

Evaluation

For each ReP the following parameters * were determined: the peak amplitude (HMAX), the latent-period (TLAT), the time-to-peak (TMAX), and the decrease-time (T2). We also determined the decline-quotient (QHN = HN/HMAX; HN measured 500 ms after HMAX) as a useful measure especially for slow declines exceeding our 5 s recording interval.

Schematic representations of the parameters are shown by the figure insets.

The response height *vs* stimulus intensity (H−I) curves were calculated by computer according to the relation

$$\mathrm{HMAX} = \frac{\mathrm{HSAT} \cdot I^n}{I_{50}^n + I^n} \qquad (1)$$

used by Pak *et al.* [10] as an arbitrary modification of the Naka Rushton relation [11].

This equation allows adjustment of the slope. The saturated response height HSAT was chosen by eye; the half saturation intensity I_{50} and the exponent n were obtained by least square deviation fit to the measured values.

The symmetric form of relation (1) in the semilogarithmic plot gives reliable values for I_{50} and r, but does not quite fit the data, especially in the low light range. The actually measured curves are asymmetric and rise more steeply from zero level. Some H−I curves were drawn by hand (figures marked by letter H at right side).

Procedure

We wanted to test the influence of extracellular calcium on light and dark adaptation of *Limulus* ventral nerve photoreceptors, and additionally study a possible calcium/sodium competition in the

* List of abbreviations:

PS	physiological (reference) saline;
ReP	receptor potential
HMAX (mV)	peak amplitude of the ReP
HSAT (mV)	saturated response amplitude
TLAT (ms)	latent-period, time from the beginning of the stimulus until the first measurable increase of the response, determined by double noise value
TMAX (ms)	time-to-peak of the response, from the beginning of the stimulus
T2 (ms)	decrease-time, time needed from the peak of the response to decrease to half of the maximal response height
QHN	decline-quotient (= HN/HMAX)
HN (mV)	after-potential, response height 500 ms after response maximum
LA	light adaptation
DA	dark adaptation
t_α, t_β (ms)	delay times between the beginning of the conditioning, light-adapting illumination and test flashes evoking responses in a state of moderate light adaptation (α) or considerable dark adaptation (β)
I_0	maximal intensity available of the stimulating light; equivalent to ca. 9×10^{16} (550 nm) photons $cm^{-2}\,s^{-1}$
I_{50}	stimulus intensity evoking half saturated response height
r (mV/log I)	steepness of response height versus log stimulus intensity curve at I_{50}, given by $r = \mathrm{HSAT} \cdot \frac{n \cdot \ln 10}{4}$
ld	logarithm to the base of 2
$[Ca^{2+}]_{ex,i}$	extracellular, intracellular calcium ion concentration

Table I. Composition of salines.

		Na^+ [mmol/l]	K^+ [mmol/l]	Ca^{++} [mmol/l]	Mg^{++} [mmol/l]	Hepes [mmol/l]	Tris [mmol/l]	Cholin [mmol/l]	Sucrose [mmol/l]	Cl^- [mmol/l]	So_4^- [mmol/l]
A	I	485	10	10	55	10	–	–	–	561	30
	II	440	10	10	55	10	–	–	270	515	30
	III	440	10	100	55	10	–	–	–	695	30
B	I	486	10	10	55	10	–	–	90	561	30
	II	486	10	40	55	10	–	–	–	621	30
C	I	463	10	10	55	–	10	–	–	613	–
	II	463	10	$4 \cdot 10^{-2}$	55	–	10	15	–	608	–
	III	46	10	$4 \cdot 10^{-2}$	55	–	10	432	–	608	–
D	I	485	10	10	55	10	–	–	–	561	30
	II	52	10	10	55	10	–	435	–	563	30
E	I	471	10	10	55	10	–	–	–	606	–
	II	418	10	$2.5 \cdot 10^{-1}$	100	10	–	–	–	623	–

action of calcium on adaptation. This lead to a sequence of test salines with different $[Ca^{2+}]_{ex}$ (varied between 40 µmol/l and 100 mmol/l), each applied in a separate group of experiments. Additionally test salines with 10% of the normal $[Na^+]_{ex}$ and with double $[Mg^{2+}]_{ex}$, together with lowered or normal $[Ca^{2+}]_{ex}$ were used.

Stimulus program

Our aim was to obtain comparable data from one photoreceptor in the state of light and dark adaptation at different test flash intensities in order to measure the intensity dependence of the RePs under two conditions of adaptation. Therefore the photoreceptors were exposed to a stimulus sequence repeated every three minutes. It consisted of a constant conditioning, light-adapting illumination of 2 s duration and saturating intensity (*i.e.* evoking a saturated response amplitude), followed by two 10 ms test flashes of identical intensity. The intensity of the test flashes was decreased, from one cycle to the next, from maximal until responses became unmeasurable, and then increased again.

The first test flash (α), which was applied after a delay time t_α (starting at the beginning of the conditioning illumination and varying between 10 and 30 s for different experiments, but constant in one experiment), evoked RePs in a defined state of moderate light adaptation. The second test flash (β) was applied after a constant interval $t_\beta = 120$ s after the beginning of the conditioning illumination and evoked RePs in a considerable state of dark adaptation (inset of Fig. 1). (The time t_α was adjusted for each preparation in such a way that the α-flash evoked RePs of about half the amplitude of the RePs caused by the β-flash, at an intensity of $I_0 \cdot 2^{-3}$ or $I_0 \cdot 2^{-4}$.)

The experiments lasted up to 4 h and were carried out in the dark without any background light. Due to the relatively long interval (10 to 30 s) between conditioning illumination and first test flash (α) only a moderate degree of light adaptation was obtained. Dark adaptation should be almost complete in our experiments, at least concerning its major initial phase. (According to Claßen-Linke and Stieve [12] ca. 90% of the dark adaptation in *Limulus* photoreceptors take place within ca. two minutes under our experimental conditions – a time corresponding to t_β, after which the second test flash was applied.) At high test flash intensities the light-adapting effect of the conditioning illumination may be slightly decreased by the preceding bright β-flash.

Since the conditions of adaptation remain constant for all experiments, they will not be repeated every time in the text, but the terms "light and dark adaptation" (LA, DA) used from now on should be understood as "moderate light adaptation" and "considerable dark adaptation".

Effect of increased osmotic pressure of the physiological saline

When the calcium concentration was lowered the osmotic pressure of the test saline was kept equal to that of the reference saline (normal PS) by addition of a corresponding quantity of NaCl or cholin

chloride. Since the osmotic pressure is higher in salines with much increased $[Ca^{2+}]$, the osmotic pressure of the respective reference salines (with normal $[Ca^{2+}]$) was adjusted by addition of the appropriate amount of sucrose (see Table I). The effect of changed osmotic pressure was tested in three experiments. Responses of photoreceptors in normal physiological saline were compared with those obtained in a reference saline with raised osmotic pressure (270 mmol/l sucrose, Table I). The average effect of increased osmotic pressure on saturation amplitude HSAT and time parameters of the ReP can be seen from the first two columns (PS, PS sucrose) of Tables II and III. The changes due to the increased osmotic pressure were small: The saturation amplitude HSAT was decreased by ca. 10 mV in the state of both light and dark adaptation. Latent period TLAT, time-to-peak TMAX and decrease time T2 were slightly longer in the reference saline with increased osmotic pressure as compared to normal PS. The changes due to the less increased osmotic pressure (90 mmol/l sucrose) as compared to the physiological reference saline are expected to be even smaller than those caused by 270 mmol/l sucrose.

Results

The effect of increased $[Ca^{2+}]_{ex}$ (100 mmol/l) on the receptor potential (3 experiments, salines A I–III)

The change of size and shape of the *receptor potentials* under the influence of 100 mmol/l $[Ca^{2+}]_{ex}$ can be seen in Fig. 1 for maximal stimulus intensity (I_0) and half saturation intensity of the light-adapted receptor ($I_{\alpha 50}$) and the dark-adapted ($I_{\beta 50}$).

In this experiment the increased $[Ca^{2+}]_{ex}$ causes a more rapid decrease of the ReP directly after its peak; the peak appears to be narrower than that of the reference ReP (dotted lines). The size of the RePs is slightly reduced in the 100 mmol/l Ca^{2+} test saline.

Response height vs stimulus intensity curves from the same experiment are shown in Fig. 2 for reference saline (with increased osmotic pressure) and test saline with increased $[Ca^{2+}]_{ex}$ in the state of dark (DA, β) and light adaptation (LA, α). This graph and the average results (Table II, column 2) show that 100 mmol/l $[Ca^{2+}]_{ex}$ causes reduced saturation amplitudes (HSAT), both in the state of DA and LA. The steepness r of the response height *vs* stimulus intensity curves is practically unchanged in the increased calcium saline as compared to the reference value.

The average half saturation intensity I_{50} is not significantly changed (slightly decreased) by $[Ca^{2+}]_{ex}$ increased to 100 mmol/l in the state of DA. In the state of LA I_{50} is shifted (by about 1 log unit) towards higher test stimulus intensities as compared to the reference LA value (Table II, Fig. 2); that is to say by the same light-adapting illumination the sensitivity is much more decreased in high than in normal $[Ca^{2+}]_{ex}$.

Average values of the *shape parameters* (TLAT, TMAX, T2, QHN) recorded in the 100 mmol/l Ca^{2+} test saline at different light intensities in the state of DA (β) and LA (α) are shown in Table III, column 3. On the whole the time parameters of RePs recorded in the state of dark adaptation are not much affected by the increase of the $[Ca^{2+}]_{ex}$: The DA values of latent-period TLAT and time-to-peak TMAX are close to their reference values, or slightly shorter, at all stimulus intensities. The DA values (β) of the decrease-time T2 are increased by high calcium conditions at I_0, but at the lower flash intensities increased $[Ca^{2+}]_{ex}$ makes them slightly shorter than their reference value.

As compared to the α-reference values (PS) of the light-adapted photoreceptor the α values of the time parameters are shorter under high calcium conditions.

At the half saturation intensity of the dark-adapted photoreceptor ($I_{\beta 50}$) there are no LA values, since α-response were too small to be detectable.

Recordings from a single experiment of time parameters *vs* flash intensity in the state of light (α-curve) and dark adaptation (β-curve) under normal conditions (PS) and under the influence of 100 mmol/l Ca^{2+} are shown in Figs. 3a, b and 4a, b.

The results are in line with the average values of Table III. The increased $[Ca^{2+}]_{ex}$ causes the curves of TLAT and TMAX to shift towards higher light intensities (both α and β values), otherwise the changes are insignificant (Fig. 3).

The values of the decrease-time T2 are shifted towards higher light intensities in the test saline with increased $[Ca^{2+}]_{ex}$. Both LA (α) and DA (β) values are considerably shortened under high calcium conditions in this experiment (Fig. 4a).

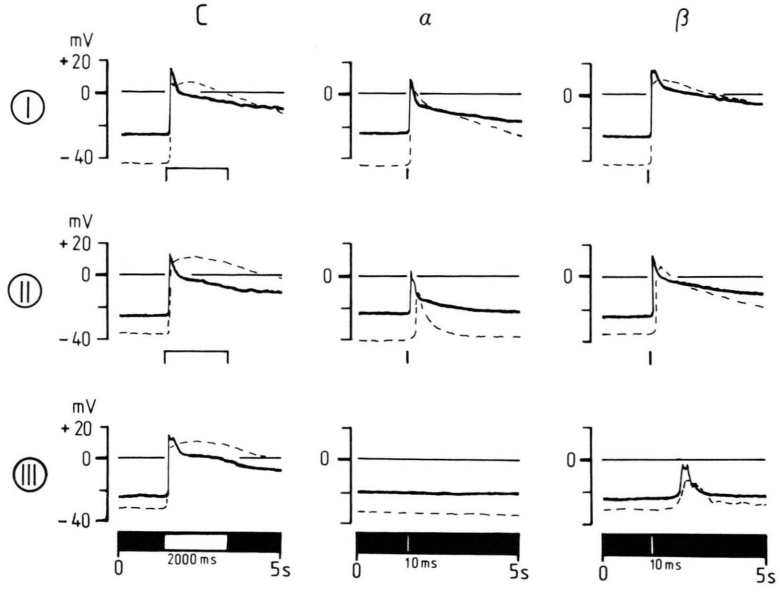

Fig. 1. Effect of raised $[Ca^{2+}]_{ex}$ (100 mmol/l) on receptor potentials of *Limulus* ventral photoreceptors at different test flash intensities and in different states of adaptation. Dashed lines: reference potentials recorded in reference saline with increased osmotic pressure. Salines AII, III, Table I. First plot in each line: response to conditioning 2 s stimulus (C, white light, intensity equivalent to ca. $1.8 \cdot 10^{16}$ (550 nm) photons $cm^{-2}\,s^{-1}$) second plots (α): LA response to α test flashes; third plots (β): DA response to β test flashes; (α and β test flashes: 10 ms white light, maximal intensity equivalent to ca. $9 \cdot 10^{16}$ (550 nm) photons $cm^{-2}\,s^{-1}$). Intensity reduced from maximal (trace I) to ca. log $I_{50\alpha}$ (II) and ca. log $I_{50\beta}$ (III). JB 223

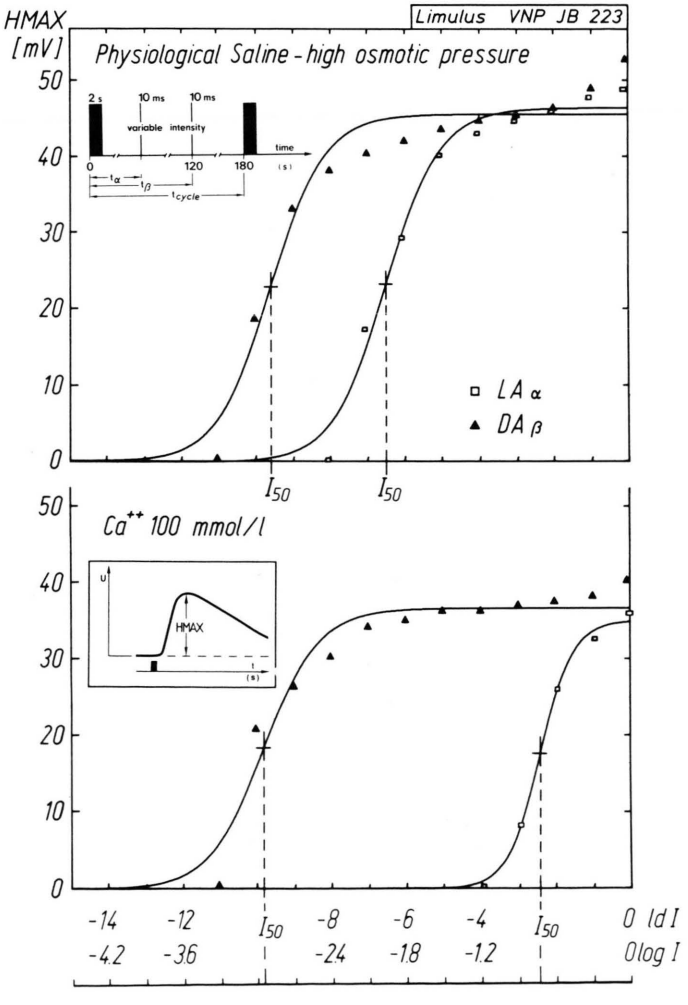

Fig. 2. Effect of raised $[Ca^{2+}]_{ex}$ (100 mmol/l) on response height (HMAX) *vs* stimulus intensity (I) curves of *Limulus* photoreceptors in the state of dark (DA, β) and light adaptation (LA, α). Upper plot: physiological saline (increased osmotic pressure); lower plot: test saline with 100 mmol/l Ca^{2+} (salines AII and AIII in Table I). Test stimulus sequence and measured parameter shown as insets. Half saturation intensity log I_{50} indicated by bars on the abscissa. Experimental conditions as in Fig. 1.

Table II. Average values from all experiments of saturation amplitude HSAT, steepness r, exponent n and half saturation intensity I_{50} of response height *vs* stimulus intensity curves in the state of light (α) and dark (β) adaptation for all reference and test salines used. Experimental conditions as described in legend of Fig. 1. Total number of experiments 15 (PS), numbers for test salines as indicated.

		1 PS	2 PS + sucrose	3 100 mmol/l Ca^{2+}	4 40 mmol/l Ca^{2+}	5 40 µmol/l Ca^{2+}	6 40 µmmol/l Ca^{2+} 46 mmol/l Na^{+}	7 48 mmol/l Na^{+}
HSAT		15	3	3	4	3	3	3
(mV)	α	51 ± 0.8	43 ± 1.3	28 ± 4.8	52 ± 8.5	52 ± 3.8	37 ± 11	25 ± 7.3
		15	3	3	5	3	3	3
	β	54 ± 2.1	45 ± 1.3	36 ± 0.7	56 ± 4.9	55 ± 6.0	42 ± 19	29 ± 6.9
r		14	3	3	4	3	3	3
(mV/log)	α	39 ± 4.0	29 ± 14	31 ± 3.9	30 ± 8.6	30 ± 6.6	37 ± 4.3	11 ± 1.1
		15	3	3	5	3	3	3
	β	33 ± 4.4	23 ± 8.3	21 ± 11	21 ± 4.7	43 ± 17	34 ± 2.7	9 ± 4.2
n		15	3	3	4	3	3	3
	α	1.6 ± 0.3	1.2 ± 0.6	2.2 ± 0.6	0.9 ± 0.2	1.0 ± 0.3	2.3 ± 1.1	1.0 ± 0.4
		15	3	3	5	3	3	3
	β	1.1 ± 0.1	0.9 ± 0.4	1.1 ± 0.6	0.6 ± 0.1	1.5 ± 0.8	1.6 ± 0.4	0.5 ± 0.2
I_{50}		15	3	3	4	3	3	3
(log)	α	−1.5 ± 0.1	−1.2 ± 0.3	−0.5 ± 0.1	−1.3 ± 0.2	−2.3 ± 0.3	−1.1 ± 0.4	−0.5 ± 0.1
		15	3	3	5	3	3	3
	β	−2.4 ± 0.1	−2.6 ± 0.2	−2.6 ± 0.1	−2.4 ± 0.2	−2.4 ± 0.2	−1.3 ± 0.4	−1.4 ± 0.1

This effect is also shown by the curves of the decline-quotient QHN. The values recorded in the high calcium test saline are parallel to, but lower than, the reference values (Fig. 4b).

Effect of 40 mmol/l $[Ca^{2+}]_{ex}$ on the receptor potential (5 experiments, salines B I, II)

The average effects of 40 mmol/l $[Ca^{2+}]_{ex}$ (Table II, column 4) are in the same direction, but weaker than those of 100 mmol/l $[Ca^{2+}]_{ex}$ concerning saturation amplitude (HSAT), steepness (r) and half saturation intensity (I_{50}) of the response height *vs* stimulus intensity curves in the state of dark (DA, β) and light adaptation (LA, α) *i.e.* HSAT, r and $I_{50\beta}$ are almost unchanged, $I_{50\alpha}$ is somewhat increased.

The response height *vs* stimulus intensity curves of a single experiment (Fig. 5) show a more reduced saturation amplitude (HSAT) and steepness of both DA (β) and LA (α) curves as compared to those recorded in the 100 mmol/l Ca^{2+} test saline (Fig. 2) and the reference saline, but the LA curve is shifted much less towards higher test flash intensities for 40 mmol/l $[Ca^{2+}]_{ex}$ than for 100 mmol/l $[Ca^{2+}]_{ex}$ but more than in the reference saline.

The shape parameters (TLAT, TMAX, T2 and QHN) are changed into the same direction, but to a smaller extent, by the 40 mmol/l Ca^{2+} test saline than by the 100 mmol/l Ca^{2+} saline (Table III, column 4).

The effect of decreased $[Ca^{2+}]_{ex}$ (40 µmol/l) and subsequently additionally decreased $[Na^{+}]_{ex}$ (46 mmol/l). (3 experiments, salines C I–III)

We compared the effect of (a) calcium deficiency alone with (b) the effect of combined calcium and sodium deficiency on the receptor potential, to find out whether the effect of calcium on adaptation is also characterized by a calcium/sodium antagonism, like the effect of calcium on the gating of the light-activated ion channels. The $[Ca^{2+}]_{ex}$ was reduced to ca. 40 µmol/l; subsequently the $[Na^{+}]_{ex}$ was reduced to ca. 10% of its normal value.

The effect of the two test salines on *size and shape of the receptor potentials* is shown in Fig. 6.

(a) Calcium deficiency (lines I, II, III) leads to a steep initial rise of the RePs. The decrease is very much slowed down, especially at maximal light intensity, for both light (α) and dark (β) adapted state. The amplitude of the RePs is not much changed.

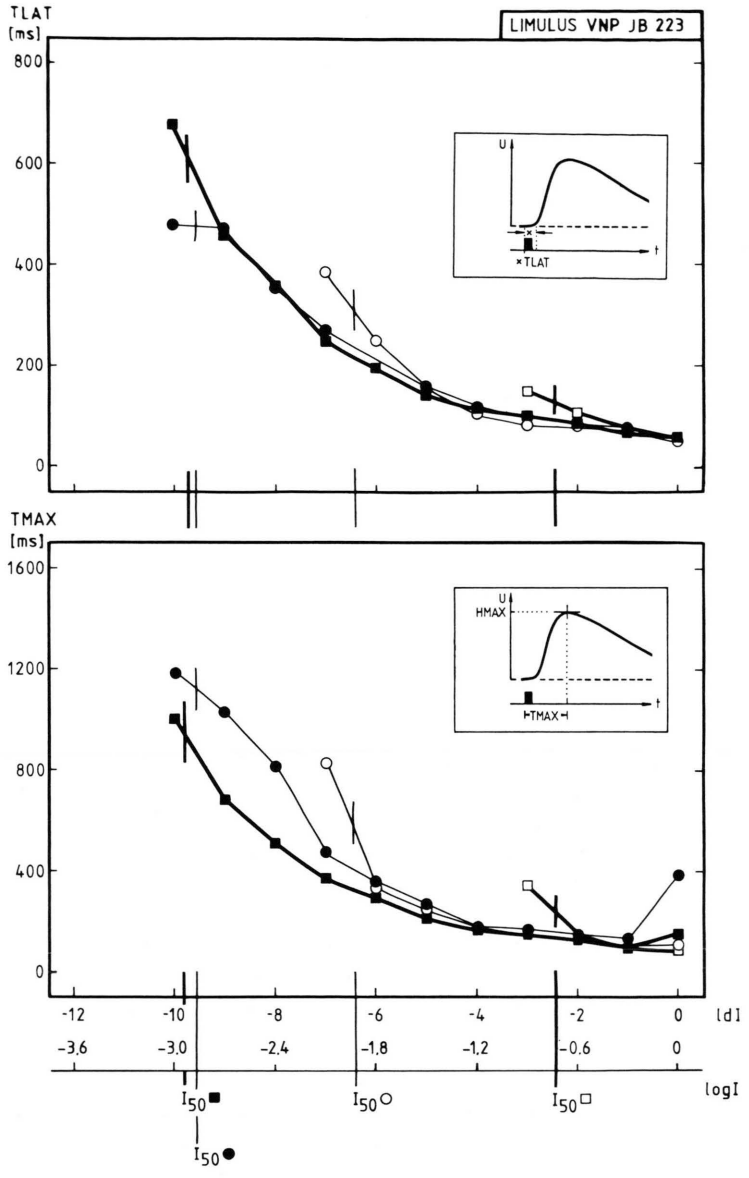

Fig. 3. Effect of raised $[Ca^{2+}]_{ex}$ (100 mmol/l) on response parameters. Latent period TLAT (upper plot) and time-to-peak TMAX (lower plot) recorded at different flash intensities in the state of dark (DA, β) and light adaptation (LA, α). Curves recorded in test saline (squares) are compared to curves recorded in reference saline with 10 mmol/l Ca^{2+} (circles). Parameters are shown in insets. I_{50}-values are marked on the abscissa. Experimental details as in Fig. 1.

(b) With additional sodium deficiency (lines IV, V, VI) the amplitude of the RePs is drastically reduced. The rising phase is much less steep, the peak of the ReP is transformed into a plateau. There is practically no decline of the ReP within our measuring interval.

(a) Calcium deficiency does not change the sensitivity of the dark-adapted photoreceptor, but it leads to a sensitivity increase of the light-adapted photoreceptor, recognizable in a marked shift of the LA (α) *curve of response height vs stimulus intensity* towards lower light intensities (second plot of Fig. 7, I_{50} values of Table II, column 5), that is to say low calcium practically abolishes the decrease in sensitivity due to the light-adapting stimulus applied. The DA (β) curve of response height *vs* stimulus intensity is steeper in calcium-deficient saline as compared to PS, while the α-curve is less steep than of the reference saline (Table II).

(b) The additional decrease of the sodium concentration causes a shift of both response height *vs* stimulus intensity curves (α and β) towards higher

Table III. Average values from all experiments of time parameters of receptor potentials in the state of light (α) and dark (β) adaptation recorded in all reference and test salines used. The values of latent-period (TLAT), time-to-peak (TMAX), decrease-time (T2) and decline-quotient (QHN) were determined from the parameter *vs* log stimulus intensity plots at three points: (1) at maximal intensity I_0 (first group); (2) at $I_{50\alpha}$ (half saturation intensity of the light-adapted photoreceptor; second group, interpolated; (3) at $I_{50\beta}$ (half saturation intensity of the dark-adapted photoreceptor; third group, interpolated. Experimental conditions as described in legend of Fig. 1. Total number of experiments 15 (PS), numbers for test salines as indicated.

		1 PS	2 PS + sucrose	3 100 mmol/l Ca^{2+}	4 40 mmol/l Ca^{2+}	5 40 µmol/l Ca^{2+}	6 40 µmol/l Ca^{2+} 46 mmol/l Na^{+}	7 48 mmol/l Na^{+}
I_0								
TLAT (ms)	α	45 ± 6.8 (15)	50 ± 0.0 (2)	65 ± 2.0 (2)	39 ± 4.5 (5)	38 ± 3.8 (3)	74 ± 28 (3)	59 ± 13 (3)
	β	53 ± 6.7 (15)	55 ± 1.5 (2)	52 ± 3.0 (2)	36 ± 6.6 (5)	43 ± 2.5 (3)	85 ± 27 (3)	56 ± 16 (3)
TMAX (ms)	α	96 ± 4.9 (15)	127 ± 11 (2)	107 ± 17 (2)	98 ± 3.5 (5)	335 ± 93 (3)	1088 ± 463 (3)	112 ± 8.6 (3)
	β	207 ± 28 (15)	306 ± 75 (2)	150 + 5.5 (2)	111 ± 7.2 (5)	677 ± 353 (3)	1765 ± 676 (3)	138 ± 5.1 (3)
T2 (ms)	α	488 ± 121 (15)	1358 ± 1162 (2)	233 ± 38 (2)	322 ± 96 (5)	2097 ± 57 (2)	1470 ± 330 (2)	89 ± 13 (3)
	β	1267 ± 170 (15)	720 (1)	1743 ± 1453 (2)	811 ± 262 (5)	2557 (1)	2175 (1)	261 ± 39 (3)
QHN	α	0.4 ± 0.05 (15)	0.5 ± 0.33 (2)	0.4 ± 0.09 (2)	0.4 ± 0.08 (5)	1.0 ± 0.01 (3)	0.9 ± 0.04 (3)	0.1 ± 0.04 (3)
	β	0.7 ± 0.04 (15)	0.8 ± 0.17 (2)	0.6 ± 0.17 (2)	0.6 ± 0.10 (5)	1.0 ± 0.02 (3)	1.0 ± 0.01 (3)	0.3 ± 0.08 (3)
$I_{\alpha 50}$								
TLAT (ms)	α	106 ± 16 (14)	192 ± 109 (2)	124 (1)	85 ± 10 (4)	117 ± 13 (3)	167 ± 51 (3)	97 ± 28 (3)
	β	113 ± 20 (15)	153 ± 73 (2)	73 ± 14 (2)	73 ± 11 (4)	184 ± 64 (3)	208 ± 64 (3)	81 ± 28 (3)
TMAX (ms)	α	263 ± 27 (14)	364 ± 156 (2)	238 (1)	192 ± 36 (4)	547 ± 81 (3)	805 ± 385 (3)	174 ± 25 (3)
	β	268 ± 36 (15)	274 ± 129 (2)	100 ± 27 (2)	147 ± 30 (4)	1130 ± 330 (3)	1361 ± 451 (3)	193 ± 39 (3)
T2 (ms)	α	143 ± 21 (14)	193 ± 47 (2)	230 (1)	89 ± 12 (4)	447 ± 32 (2)	466 ± 236 (2)	89 ± 12 (3)
	β	362 ± 67 (15)	1354 ± 827 (2)	789 ± 501 (2)	272 ± 62 (4)	415 ± 235 (2)	440 (1)	235 ± 28 (3)
QHN	α	0.1 ± 0.03 (12)	0.1 ± 0.10 (2)	0.3 (1)	0.1 ± 0.02 (4)	0.5 ± 0.08 (3)	0.6 ± 0.22 (3)	0.01 (1)
	β	0.3 ± 0.05 (15)	0.6 ± 0.11 (2)	0.5 ± 0.09 (2)	0.3 ± 0.07 (4)	0.8 ± 0.14 (3)	0.8 ± 0.18 (3)	0.2 ± 0.09 (2)

Table III (continued).

		1 PS		2 PS + sucrose		3 100 mmol/l Ca^{2+}		4 40 mmol/l Ca^{2+}		5 40 µmol/l Ca^{2+}		6 40 µmol/l Ca^{2+} 46 mmol/l Na^{+}		7 48 mmol/l Na^{+}	
$I_{\beta 50}$															
TLAT (ms)	α	866 ± 673	4	–		–		–		95 ± 17	3	176 ± 36	3	68	1
	β	236 ± 39	15	469 ± 3.5	2	504 ± 131	2	183 ± 38	5	170 ± 66	3	220 ± 51	3	181 ± 99	3
TMAX (ms)	α	1038 ± 660	4	–		–		–		540 ± 79	3	842 ± 368	3	151	1
	β	595 ± 81	15	959 ± 148	2	669 ± 257	2	443 ± 70	5	1226 ± 270	3	1325 ± 433	3	336 ± 133	3
T2 (ms)	α	79 ± 13	3	–		–		–		446 ± 16	2	375 ± 155	2	143	1
	β	242 ± 38	15	316 ± 205	2	164 ± 76	2	171 ± 35	5	1178 ± 463	2	999 ± 559	2	190 ± 45	3
QHN	α	0.1 ± 0.05	4	–		–		–		0.5 ± 0.09	3	0.5 ± 0.20	3	0	1
	β	0.3 ± 0.06	12	0.6	1	0.4 ± 0.11	2	0.1 ± 0.03	5	0.8 ± 0.10	3	0.8 ± 0.18	3	0.1 ± 0.08	2

light intensities, *i.e.* a decrease in sensitivity (Fig. 7, third plot, Table II, column 6). The saturation amplitudes (HSAT) are reduced, the α-value (LA) more than the β-value (DA). The curves (α and β) becomes much less steep (about the same steepness as in the reference saline).

(a) The effect of calcium deficiency on the *time parameters* of the response (Table III, column 5, and Figs. 8 and 9) is this: The latent period TLAT is, on the average, slightly shorter at I_0, slightly longer at $I_{\alpha 50}$ and much shorter at $I_{\beta 50}$ than the reference values, both for DA and LA. A similar tendency is shown in the single experiment, where the low calcium values (α and β) of TLAT become increasingly shorter than the reference values with decreasing flash intensity. The *time-to-peak* TMAX is longer in the calcium-deficient saline than in PS, especially at the highest light intensity applied (Table III, column 5). At $I_{\beta 50}$ the α-value of TMAX is smaller than the reference value. In the single experiment (Fig. 8) the β-values (DA) in the calcium deficient saline are longer than the reference values at lower flash intensities, while the α-values are practically identical. The *decrease-time* T2 is considerably prolonged in all cases (average values Table II, column 5, single experiment Fig. 9) for LA (α) and DA (β) in the calcium-deficient saline. This is also expressed by values of the decline-quotient QHN close to 1, which indicate an extremely slow decrease.

(b) Additional reduction of the sodium concentration to 10% (Table III, column 7, Figs. 8 and 9) causes a prolongation of latent-period TLAT and time-to-peak TMAX in both states of adaptation as compared to their previous values in the calcium deficient saline with normal sodium concentration. The α and β-values of the decrease-time T2 and the decline-quotient QHN are not significantly changed by additional sodium deficiency.

These experiments show that while (a) calcium deficiency prevents light adaptation, (b) additional sodium reduction does not counteract specifically this effect.

The effect of sodium deficiency (10% Na^{+}) alone on the receptor potential (3 experiments, saline D I, II)

Since in the previous experiments a test saline with combined calcium and sodium deficiency had been used we made three experiments where only the

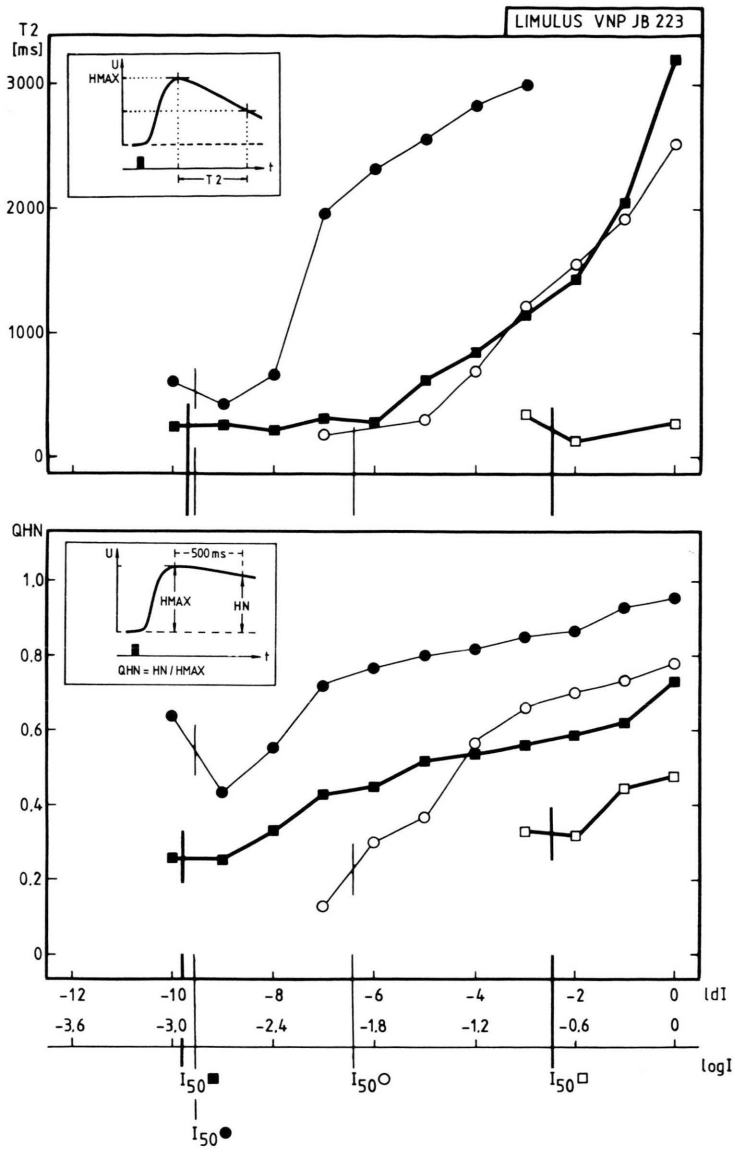

Fig. 4. Effect of raised $[Ca^{2+}]_{ex}$ (100 mmol/l) on response parameters. Decrease-time T2 (upper plot) and decline-quotient QHN (lower plot) recorded at different flash intensities in the state of dark (DA, β) and light adaptation (LA, α). Further description as in Fig. 3. Experimental details as in Fig. 1.

$[Na^+]_{ex}$ was reduced (to 10%), while the $[Ca^{2+}]_{ex}$ was normal.

Sodium deficiency leads to a sensitivity decrease of both dark and light adapted photoreceptors by about the same amount (shift of I_{50} of ca. 1 log unit in both response height *vs* stimulus intensity curve of Fig. 10).

Both LA (α) and DA (β) values of the saturated response height HSAT are reduced to ca. half their reference values (Table II, column 7) by sodium deficiency.

The steepness r of the α and β curves is much smaller due to the lack of sodium.

The effect of sodium deficiency on the LA curve could have been partly due to reduced speed of the dark adaptation following the conditioning illumination as a consequence of the reduced sodium concentration as observed by Wulff *et al.* [13] and Fein and Charlton [14]. In other words, a longer interval t_α might compensate in these experiments for an adaptation delay: Owing to our fixed flash sequence we could not test this possibility directly

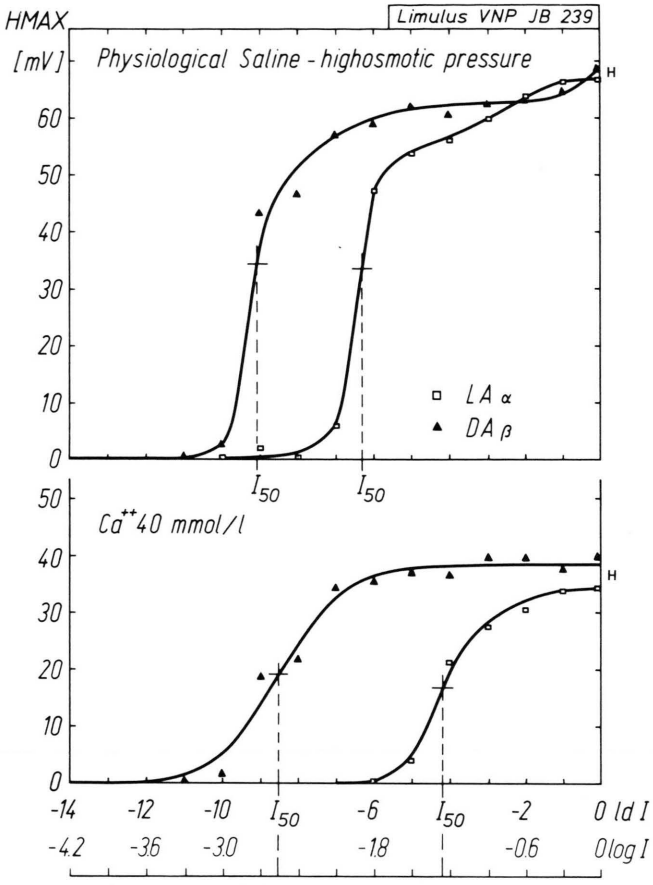

Fig. 5. Effect of raised $[Ca^{2+}]_{ex}$ (40 mmol/l) on response-height (HMAX) *vs* stimulus intensity (I) curves of *Limulus* ventral photoreceptors in the state of dark (DA, β) and light adaptation (LA, α). Upper plot: reference saline (10 mmol/l Ca^{2+}, increased osmotic pressure); lower plot: test saline with 40 mmol/l Ca^{2+} (salines BI, II). I_{50}-values are marked on the abscissa. Experimental details as in Fig. 1. Curves drawn by hand.

in the same experiment, but previous experiments, where t_α was varied (between 2 s and 20 min) showed that the saturation amplitude of the light-adapted photoreceptors in a sodium-deficient saline remains at a lower level than the reference value, while the adaptation took about the same time. This means that the interval t_α does not much influence the results.

The main effect of sodium deficiency on the time parameters of the ReP (average values, Table III, column 7) is a marked shortening of the decrease-time T2 (and a reduction of the decline-quotient QHN) both for DA (β) and LA (α) values. While at maximal test flash intensities (I_0) α and β values of latent-period TLAT and time-to-peak TMAX are the same in the sodium-deficient saline than under normal conditions, they become shorter than their reference values at lower test flash intensities under low sodium conditions.

The effect of increased $[Mg^{2+}]_{ex}$ (100 mmol/l) and decreased $[Ca^{2+}]_{ex}$ (250 µmol/l) on the receptor potential (salines E I, II)

Magnesium ions can replace calcium, though with a weaker action, in its effect on the function of the light-induced increase in membrane conductance [7].

We therefore tested whether the effect of lowering the $[Ca^{2+}]_{ex}$ on light adaptation can also be counteracted by raising the $[Mg^{2+}]_{ex}$ of the test saline.

These experiments (three, with basically similar results; here only one is described, in which the degree of light and dark adaptation was the same as in the experiments with the other test salines) showed that the dark-adapted (β) curve of *response height vs stimulus intensity* recorded in the high

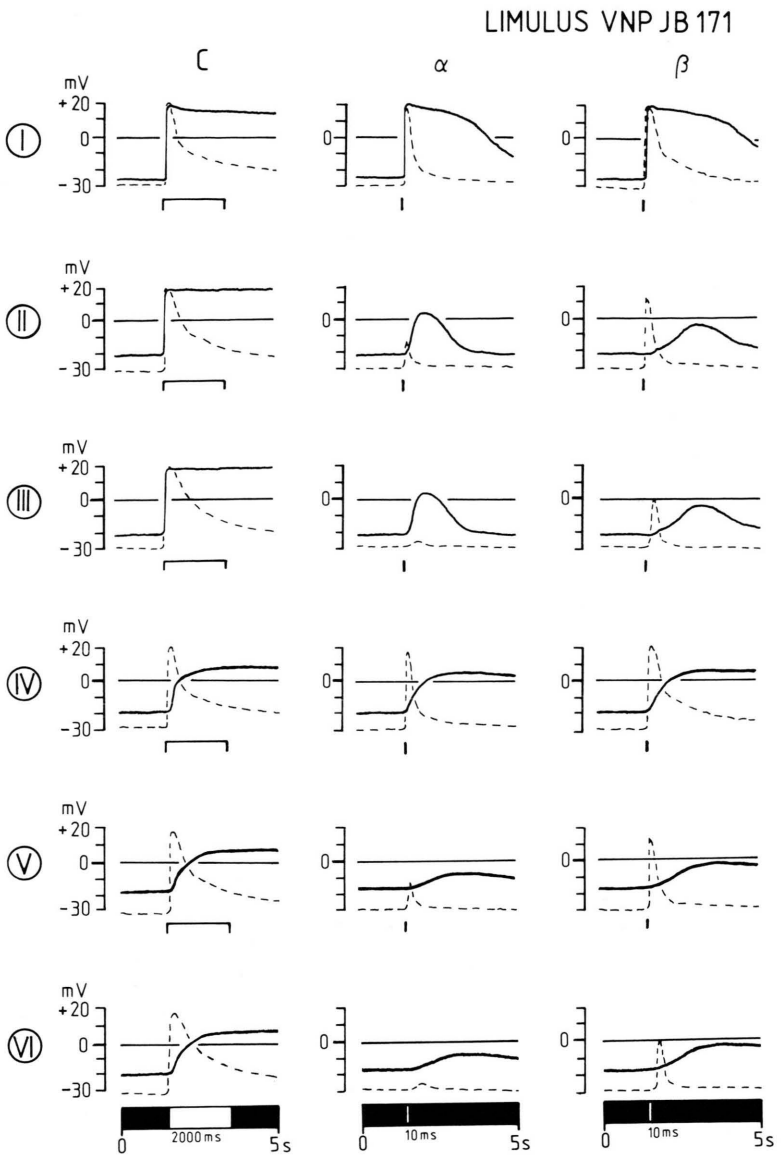

Fig. 6. Effect of reduction of the $[Ca^{2+}]_{ex}$ from 10 mmol/l to 40 µmol/l and subsequently additional reduction of the $[Na^{+}]_{ex}$ from 463 mmol/l to 46 mmol/l on receptor potentials at different test flash intensities and in different states of adaptation. First plot in each line: response to conditioning 2 s stimulus (C); second plots (α): LA response to α test flashes; third plots: DA responses to β test flashes. Line I to III: test saline with 40 µmol/l Ca^{2+}; Line IV to VI: test saline with 40 µmol/l Ca^{2+} and 46 mmol/l Na^{+}. Salines CI, II, III. Test flash intensity reduced from maximal (lines I and IV) to ca. log $I_{50\alpha}$ (lines II and V) and ca. log $I_{50\beta}$ (lines III and VI). Responses to α and β test flashes identical in this experiment. Dashed lines: reference potentials recorded in physiological saline with increased osmotic pressure. Experimental details as in Fig. 1.

magnesium test saline was shifted to higher stimulus intensities, whereas the light-adapted α-curve was shifted to lower intensities. Fig. 11 shows almost identical α and β curves recorded in the test saline with indistinguishable values of I_{50}. The saturation amplitudes (Fig. 11, Table II) recorded in the test saline are slightly higher than the reference values.

All *time parameters* are prolonged in the test saline with increased $[Mg^{2+}]_{ex}$ and lowered $[Ca^{2+}]_{ex}$. The latent-period is slightly and the time-to-peak much increased.

The decline of the ReP is very much slowed down (two-fold increase of T2 at I_0, ten-fold increase at $I_{\beta 50}$ as compared to reference values). This is also demonstrated by the decline-quotient, which rises up to values close to 1.

The results show that a calcium decrease prevents a sensitivity shift due to light adaptation. This effect is not counteracted by an increase in the $[Mg^{2+}]_{ex}$. The sensitivity of the dark-adapted photoreceptor is reduced by an increase of the $[Mg^{2+}]_{ex}$ (shift of the β-curve, Fig. 11).

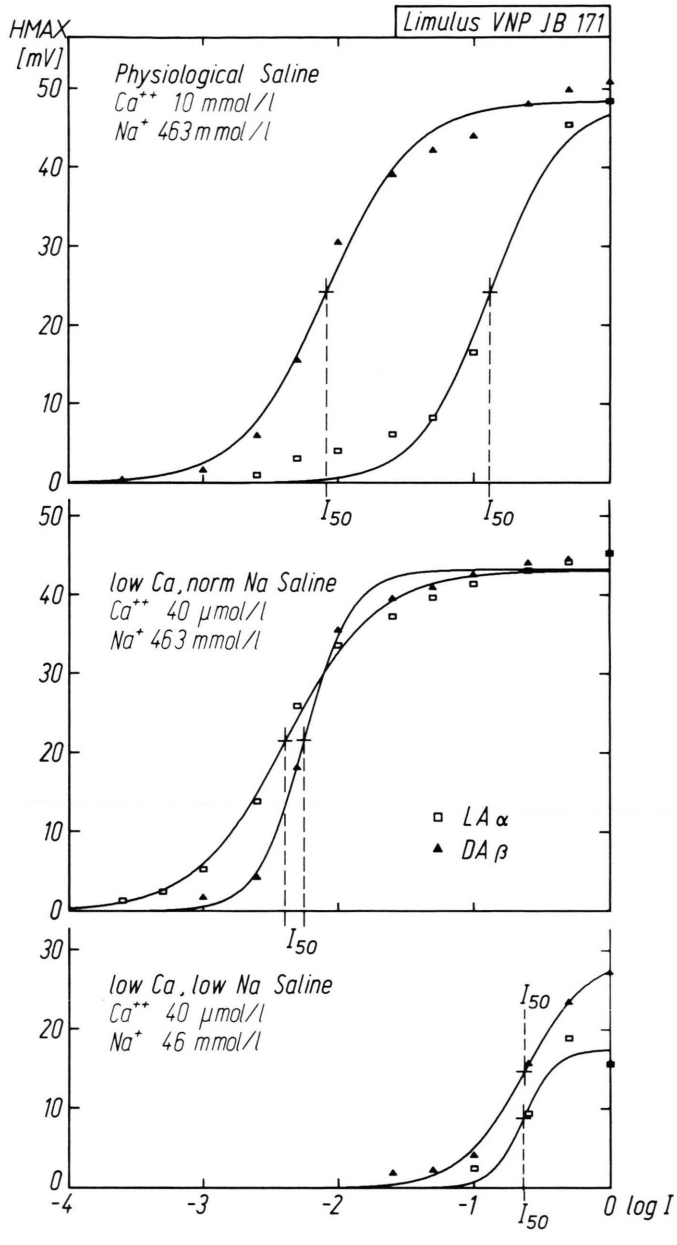

Fig. 7. Effect of reduction of the $[Ca^{2+}]_{ex}$ from 10 mmol/l to 40 µmol/l and subsequently additional reduction of the $[Na^+]_{ex}$ from 463 mmol/l to 46 mmol/l on response height HMAX *vs* stimulus intensity curves of *Limulus* ventral photoreceptors in the state of dark (DA, β) and light adaptation (LA, α). First plot: physiological saline; second plot: test saline with 40 µmol/l Ca^{2+}; third plot: test saline with 40 µmol/l Ca^{2+} and 46 mmol/l Na^+ (salines CI, II, III). I_{50} values are marked on the abscissa. Experimental details as in Fig. 1.

Magnesium ions cannot replace calcium ions. On the contrary, the effect of lowering the $[Ca^{2+}]_{ex}$ (prolongation of T2 and reduction of the sensitivity shift due to light adaptation) seems to be even enhanced by the raised $[Mg^{2+}]_{ex}$.

The effect of different calcium (and sodium) concentrations on the dark- and light-adapted photoreceptor

The stimulus intensity I_{50} which evokes half saturation of the response amplitude obtained from response height *vs* stimulus intensity curves of light and dark-adapted photoreceptors in test salines with different calcium concentrations can be taken as a measure to characterize and compare the sensitivity of the visual cells. We therefore plotted the half saturation intensities measured in our experiments *vs* the $[Ca^{2+}]_{ex}$ in a double logarithmic scale (Fig. 12, which is a graphic illustration of the I_{50}-values from Table II). The half saturation intensity of the dark-adapted photoreceptor is practically not influenced by calcium deficiency and calcium increase up to 40 mmol/l. Only the concentration of 100 mmol/l

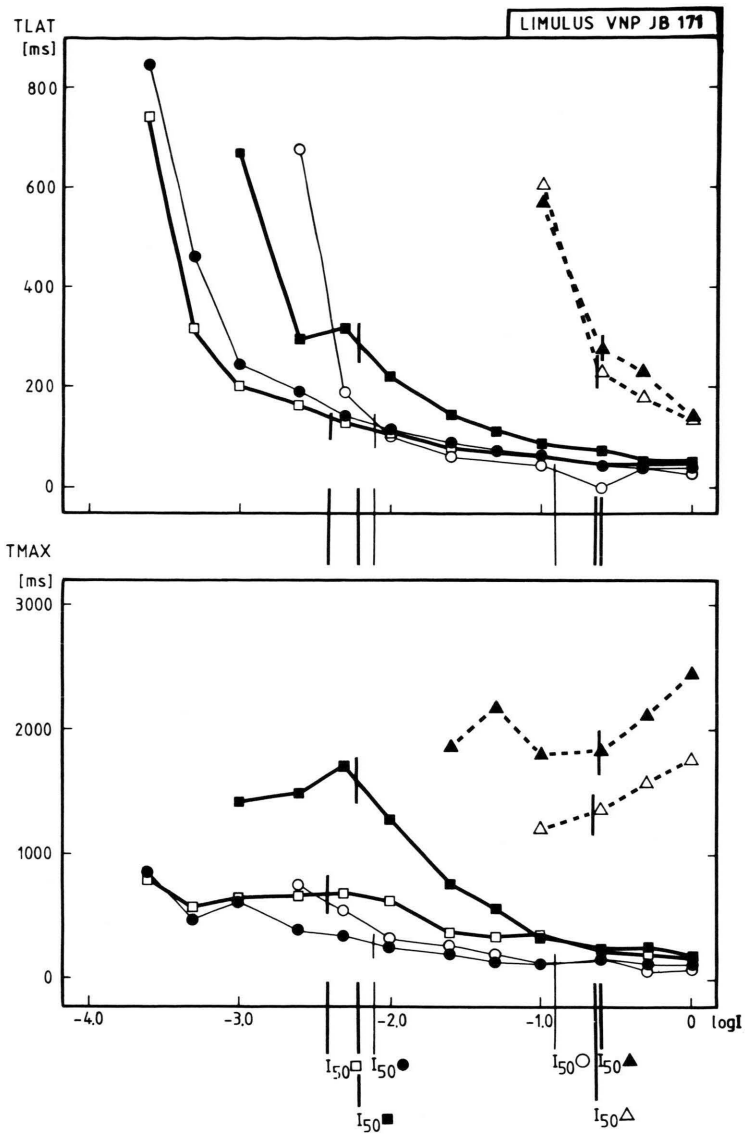

Fig. 8. Effect of reduction of the $[Ca^{2+}]_{ex}$ from 10 mmol/l to 40 µmol/l and subsequently additional reduction of the $[Na^{+}]_{ex}$ from 463 mmol/l to 46 mmol/l on response parameters. Latent period TLAT (upper plot) and time-to-peak TMAX (Lower plot) recorded at different flash intensities in the state of dark (DA, β) and light adaptation (LA, α). Curves recorded in low calcium saline (squares) and low calcium, low sodium saline (triangles) are compared with curves recorded in reference saline (circles). I_{50} values are marked on the abscissa. Experimental details as in Fig. 1.

causes a slight change of the half saturation intensity to lower light intensities (sensitivity increase).

Opposite to this almost negligible effect on the dark-adapted photoreceptor the $[Ca^{2+}]_{ex}$ has a major influence on the light-adapted photoreceptor. The extent of light adaptation (caused by identical conditioning illuminations) decreases with calcium deficient saline and increase significantly with rising $[Ca^{2+}]_{ex}$.

In other words: the receptor becomes the more desensitized by light-adapting illuminations the more calcium ions are present in the external saline in the concentration range applied in our experiments.

The half saturation intensity values of response height *vs* stimulus intensity curves recorded in a combined calcium and sodium deficient saline (upper plot) are shifted parallel to those obtained in a saline where only the $[Ca^{2+}]_{ex}$ was changed, both for dark and light adaptation. Sodium deficiency shifts the sensitivity of the photoreceptor towards higher light intensities irrespective of the changes brought about by the influence of the calcium concentration, both for the LA and the DA curve.

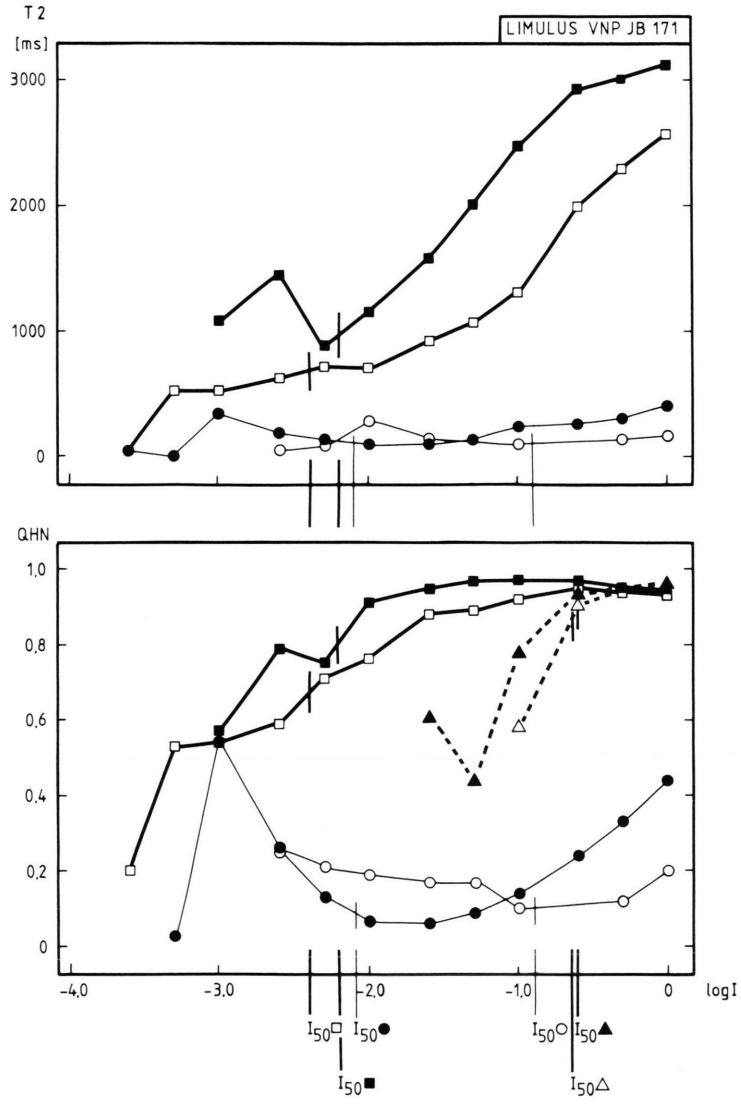

Fig. 9. Effect of reduction of the $[Ca^{2+}]_{ex}$ from 10 mmol/l to 40 µmol/l and subsequently additional reduction of the $[Na^{+}]_{ex}$ from 463 mmol/l to 46 mmol/l on response parameters. Decrease-time T2 (upper plot) and decline-quotient QHN recorded at different flash intensities in the state of dark (DA, β) and light adaptation (LA, α). Further description as in Fig. 8. Experimental details as in Fig. 1.

The effect of external calcium concentration is not characterized by a calcium/sodium antagonism, in contrast to the effect of calcium on the gating of the light-activated ion channels.

An increase of the external magnesium concentration seems to augment the effects of calcium deficiency, and certainly does not counteract the calcium lack.

Discussion

The receptor potential of the *Limulus* ventral photoreceptor is primarily composed of superimposed bumps and only secondarily modified by voltage-sensitive membrane conductances. Under conditions close to normal (physiological saline, no background illumination) a size reduction of individual bumps is the main cause of the change of the macroscopic light response, the ReP, due to light adaptation [15, 1], which is more pronounced for the decline phase of the receptor potential, and a narrowing of the latency distribution and shortening of the average latent period due to light adaptation. The effects of changed $[Ca^{2+}]_{ex}$ can be explained on the same basis. Low $[Ca^{2+}]_{ex}$ broadens the bump latency distribution [16], which results mainly in a

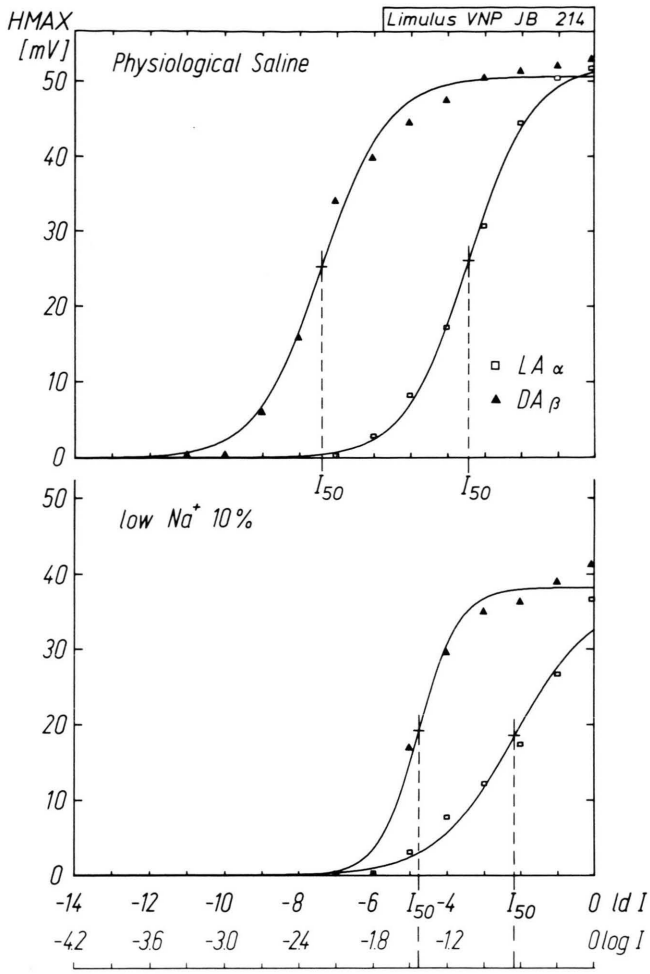

Fig. 10. Effect of reduction of the $[Na^+]_{ex}$ from 463 mmol/l to 46 mmol/l on response height (HMAX) *vs* stimulus intensity (I) curves of *Limulus* photoreceptors in the state of dark (DA, β) and light adaptation (LA, α); $[Ca^{2+}]_{ex}$ unchanged (10 mmol/l). Upper plot: physiological saline; lower plot: test saline with 46 mmol/l Na^+ (salines DI, II). I_{50} values are marked on the abscissa. Experimental details as in Fig. 1.

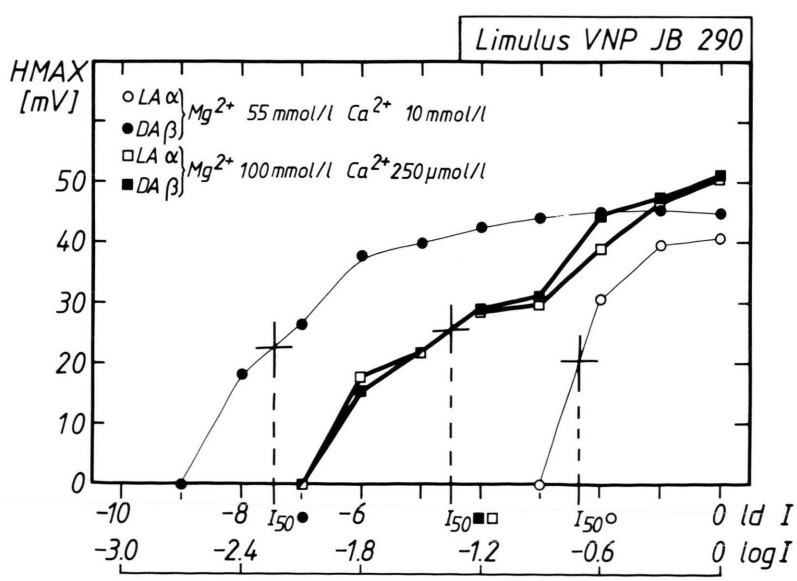

Fig. 11. Effect of reduced $[Ca^{2+}]_{ex}$ (250 µmol/l) and raised $[Mg^{2+}]_{ex}$ (100 mmol/l) on response height (HMAX) *vs* stimulus intensity (I) curves of *Limulus* photoreceptors in the state of dark (DA, β) and light adaptation (LA, α), as compared to curves obtained in reference saline (10 mmol/l Ca^{2+}, 55 mmol/l Mg^{2+}). Salines EI, II. Log I_{50} values are marked on the abscissa (PS, α curve: -0.71, β curve: -2.74; test saline, α and β curve: -1.35). HSAT: PS, α-value 41 mV, β-value 45 mV; test saline α-value 50 mV, β-value 51 mV. Steepness r: PS, α-value 97 mV/log, β-value 33 mV/log; test saline, α-value 14.5 mV/log, β-value 21.5 mV/log. Experimental details as in Fig. 1.

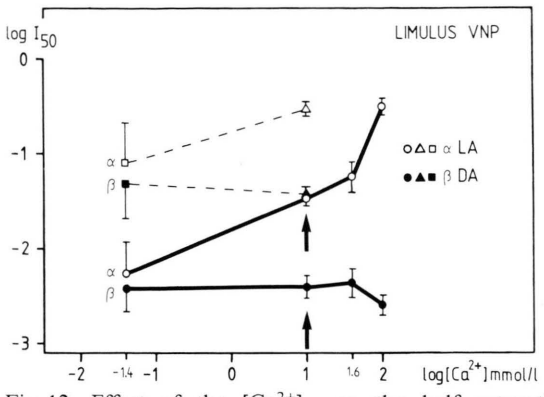

Fig. 12. Effect of the $[Ca^{2+}]_{ex}$ on the half saturation intensity I_{50} of response height *vs* stimulus intensity curves of *Limulus* ventral photoreceptors in the state of dark (β, solid circles) and light adaptation (α, open circles). Experiments with reduced $[Na^+]_{ex}$ (46 mmol/l) and normal $[Ca^{2+}]_{ex}$ are shown by solid triangles (DA) and open triangles (LA). Experiments with reduced $[Na^+]_{ex}$ (48 mmol/l) and reduced $[Ca^{2+}]_{ex}$ (40 µmol/l) are shown by solid squares (DA) and open squares (LA). Experimental details as in Fig. 1.

prolongation of the decrease-time of the ReP. High $[Ca^{2+}]_{ex}$ narrows the bump latency distribution, thus shortening the decrease time. This decrease time is additionally shortened, since under high calcium conditions light adaptation is stronger and therefore the tail of the ReP is composed of smaller (light-adapted) bumps. The intensity dependence of the dark adapted receptor potential is not much changed by different $[Ca^{2+}]_{ex}$. Light adaptation (bump size reduction) is mainly dependent on calcium influx, which does not take place when the $[Ca^{2+}]_{ex}$ is very low.

Injection of Ca^{2+} ions into *Limulus* ventral photoreceptors mimics the desensitizing effect of light adaptation, while injection of the calcium buffer EGTA opposes light adaptation [3, 4]. Brown and Blinks [17] demonstrated a transient light-induced calcium ion concentration increase (by injecting the Ca^{2+} indicator aequorin) in *Limulus* photoreceptors.

Similar results were obtained (with Arsenazo III as Ca^{2+} indicator) by Brown *et al.* [18], Maaz and Stieve [19], Nagy and Stieve [20], and Ivens and Stieve [21].

A substantial part of the intracellular calcium increase comes from extracellular sources [19, 21].

Micro-injection of calcium into *Limulus* photoreceptors has the same desensitizing effect as illumination by a light spot [22, 5].

Our results clearly indicate that the light-adapting effect of identical conditioning illuminations increases with increasing $[Ca^{2+}]_{ex}$ (see Fig. 12). In other words, the higher the $[Ca^{2+}]_{ex}$, the more intensely the photoreceptor is light-adapted.

Contrary to this effect on light adaptation the $[Ca^{2+}]_{ex}$ has practically no effect on the response height *vs* stimulus intensity curve of the dark-adapted photoreceptor. If adaptation is explained by changes in the $[Ca^{2+}]_i$ apparently the $[Ca^{2+}]_i$ is kept on a more or less constant low level in the dark, no matter how the $[Ca^{2+}]_{ex}$ is varied (within 40 µmol/l and 100 mmol/l). A slight deviation (sensitivity increase) was recorded for the dark adapted photoreceptor when the $[Ca^{2+}]_{ex}$ was 100 mmol/l. This is in agreement with the assumption that light adaptation is based on an increase of the intracellular calcium concentration from extracellular sources. During dark adaptation the intracellular calcium concentration is lowered by processes (presumably active transport across the cell membrane [21]) which do not depend as much on $[Ca^{2+}]_{ex}$.

In very low $[Ca^{2+}]_{ex}$ ($\leqq 10^{-6}$ mmol/l) the light-activated ion channels are permanently open (and therefore inexcitable by light) even in the dark due to extracellular calcium deficiency [6]. This can be prevented by additionally lowering the $[Na^+]_{ex}$ only to 10% of its normal concentration.

This effect of low $[Ca^{2+}]_{ex}$ on gating of the light activated ion channels is distinctly different from that on light adaptation for several reason: It occurs at much lower $[Ca^{2+}]_{ex}$, can be partly counteracted by raising the external magnesium ion concentration and it is characterized by a calcium/sodium antagonism, while the effect of low $[Ca^{2+}]_{ex}$ on light adaptation can not be counteracted by additionally lowering the $[Na^+]_{ex}$ or raising the $[Mg^{2+}]_{ex}$.

There are (at least) two different effects of low $[Ca^{2+}]_{ex}$ on the photoreceptor, distinguished by their topographically different sites of action:

(a) Extracellular calcium deficiency leads to the opening of light-activated ion channels; this effect can be partly reversed by additionally lowering the $[Na^+]_{ex}$ or raising the $[Mg^{2+}]_{ex}$ [7]. The site of this action of calcium is presumably at the outer surface of the photosensory membrane.

(b) Low $[Ca^{2+}]_{ex}$ reduces the extent of light adaptation (during the first, fast phase of light adaptation measured in our experiments [12]). This

intracellular effect of low calcium (which is not characterized by calcium/sodium antagonism and cannot be opposed by increased $[Mg^{2+}]_{ex}$) can be explained by the assumption that calcium ions from extracellular sources enter the photoreceptor cell upon stimulation by light, whereas the $[Ca^{2+}]_i$ in the dark is not much dependent of external $[Ca^{2+}]$, [21, 23]. Perhaps here magnesium ions also compete for the same site as calcium ions, however with very weak action or none at all, which would explain that raised $[Mg^{2+}]_{ex}$ seems to augment the effect of calcium deficiency.

Acknowledgements

We thank A. Eckert and M. Fürst for typing tables and manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft, SFB 160.

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Note added in proof

1) In the experiments with low calcium concentration in te superfusate (as in Fig. 7) the intensity dependence curve of the receptor potential amplitude of the dark adapted photoreceptor cross that of the light adapted receptor. This phenomenon is already described and discussed under the aspect of "facilitation" in [24].

2) Experiments in which the light intensity dependence of the light-induced membrane current of the (voltage clamped) *Limulus* ventral nerve photoreceptor was measured for 3 external Ca^{2+}-concentrations (0.25, 10 and 40 mmol/l [23]), gave results, which are in good agreement with those described here.