Calcium/Sodium Binding Competition in the Gating of Light-Activated Membrane Conductance Studied by Voltage Clamp Technique in *Limulus* Ventral Nerve Photoreceptor

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Z. Naturforsch. **40 c**, 278–291 (1985); received November 19, 1984

Limulus Ventral Nerve Photoreceptor, Current-Voltage Relation, Varied External Calcium and Sodium Concentration, Calcium/Sodium Binding Competition, Lithium

The membrane current *vs.* voltage dependence was measured in *Limulus* ventral nerve photoreceptors at various external Ca^{2+} and Na^{+} concentrations, using the voltage clamp technique. Lowering the external concentration of the divalent cations Ca^{2+} and Mg^{+} to $<1\,\mu$mol/l by adding EDTA causes

1) the light-induced transient conductance increase to disappear and
2) $V_{rev}J_D$, the reversal potential of the membrane current in the dark to shift to a positive value between +10 and +20 mV. This value is about the same as the ($V_{rev}J_L$), reversal potential of the total light current under normal ionic conditions.

If the external Na^{+} is lowered to 50 mmol/l (*i.e.* 10% of the normal concentration) simultaneously with the lowering of the divalent cation concentration described above, the light response is not abolished and $V_{rev}J_D$ is shifted less. The extent of this antagonism depends on the sodium substitute; it is stronger if choline is used instead of lithium.

Lowering of sodium alone to 50 mmol/l, in a saline containing normal Ca^{2+} and Mg^{2+} concentrations, does not change the membrane dark current *vs.* voltage curve and so $V_{rev}J_D$ is not altered; $V_{rev}\Delta J_L$, the reversal potential of the light-induced current, however, is reduced by 10 mV (from +20 to +10 mV). This reduction in $V_{rev}\Delta J_L$ can be accounted for by the reduction of the sodium gradient across the cell membrane.

Raising the external Ca^{2+} concentration to 40 or 100 mmol/l has no conspicuous effect on the membrane current *vs.* voltage dependence and the gating of the light-induced conductance increase.

The results are consistent with our working hypothesis that the gating of the light-activated ion channels in *Limulus* photoreceptor is controlled by negative binding sites for which calcium- and sodium ions compete with antagonistic actions.

Abbreviations: ReP, receptor potential (membrane voltage response to the light stimulus); HMAX [mV], peak amplitude of the ReP; PMP [mV], pre-stimulus membrane potential (difference between the extracellularly recorded zero line and the intracellularly recorded base line; initial and final zero were averaged to compensate for drift); V_M [mV], membrane voltage; J_M [nA], membrane current; J_D [nA], dark current, membrane current during voltage clamp, measured 1 s after clamp onset; J_L [nA], amplitude of total light current; amplitude of total membrane current following the light flash ($J_D+\Delta J_L$); ΔJ_L, amplitude of light-induced current; $V_{rev}J_D$ [mV], reversal potential of dark current; $V_{rev}J_L$ [mV], reversal potential of light current; $V_{rev}\Delta J_L$ [mV], reversal potential of the light-induced current evoked by the light stimulus; g_D [μS], membrane (slope) conductance in the dark; g_L [μS], total membrane (slope) conductance during illumination; Δg_L [μS], light induced membrane (slope) conductance; PS, physiological saline (see Table I); $[Na^+]_{ex}$ $[Ca^{2+}]_{ex}$, sodium, or calcium ion concentration of the external saline; EDTA, ethylene dimethyl tetra acetic acid; EGTA, ethylene glycol-bis (2-aminoethylether) N,N′-tetra acetic acid.

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0341-0382/85/0300-0278 $ 01.30/0

Introduction

In a previous publication [1] we described a working hypothesis of a calcium/sodium binding competition controlling the gating of the light-activated ion current in the photosensory membrane of the photoreceptor of *Limulus:* the membrane potential in the dark is depolarized when the external concentration of divalent cations (calcium and magnesium) is reduced. At very low calcium and magnesium concentrations ($<10^{-6}$ mol/l) the dark potential is reversed to positive values and the light-induced receptor potential is very much reduced or even abolished. Additional lowering of the sodium concentration (to 50 mmol/l) leads to a substantial recovery of both membrane potential in the dark and light response [1, 2].

The effect of varying the external calcium, magnesium and sodium concentration in arthropod

photoreceptors has been studied by several authors: Reduction of calcium to concentrations less than 10^{-5} mol/l (in the presence of magnesium) generally causes only a slight decrease of the membrane potential in the dark, but a significant increase in the amplitude of the light response [3–9]. The light-induced membrane current becomes larger, the reversal voltage of the light-induced current is not changed [3, 7, 10]. Brown and Blinks [11] observed, in *Limulus* ventral nerve photoreceptors, that in a calcium and magnesium deficient saline (with EGTA) the membrane voltage in the dark becomes positive and approaches (or even exceeds) the reversal potential for the light response within 1–2 min. Voltage clamp measurements still showed a large light-induced membrane conductance change in this condition. Lowering the external sodium concentration is reported not to influence the membrane potential in the dark [3, 6, 8], or to decrease it somewhat [12]. The light response becomes smaller [4, 8, 13] and the reversal potential of the light-induced current is reported to be somewhat decreased [3, 7, 10].

Based on our results with *Limulus* and other invertebrate photoreceptors we formulated a working hypothesis (Fig. 1) assuming that opening and closing of the light-activated ion channels in the visual cell membrane is controlled by negative binding sites for which calcium and sodium ions compete with an antagonistic action [1, 14]. Our model, apart from the calcium-antagonistic role of sodium, has several features in common with the model proposed by Weeks and Duncan [15] for the cephalopod retina. A calcium/sodium antagonism was also observed by Brown *et al.* [3] in the barnacle photoreceptor. Besides some striking similarities between their model and ours, there is one difference: our assumption that light channels are opened in the dark due to a lowering of the external concentration of divalent cations, derived from our observations with much lower divalent cation concentrations than those used by these authors. Brown and Ottoson [16] observed that extracellular potassium ions counteract the action of extracellular calcium in the barnacle photoreceptor. We could not confirm this calcium/potassium antagonism in *Limulus* and crayfish photoreceptors [17].

To further test our hypothesis we made the voltage clamp experiments reported here. Using two intracellular microelectrodes we measured, in *Limulus* ventral nerve photoreceptor cells, the membrane current in the dark J_D, the light current J_L, and the light-induced membrane current ΔJ_L, depending on the membrane voltage, in salines with varied ionic composition.

The concentration of calcium and magnesium ions was lowered from normal to ca. 1 µmol/l (by using the calcium-chelating agent EDTA), or raised to 40 or 100 mmol/l; the concentration of sodium ions was reduced to 50 mmol/l (10% of its normal value).

The results (some of which have been briefly published before [18]) are in agreement with our working hypothesis.

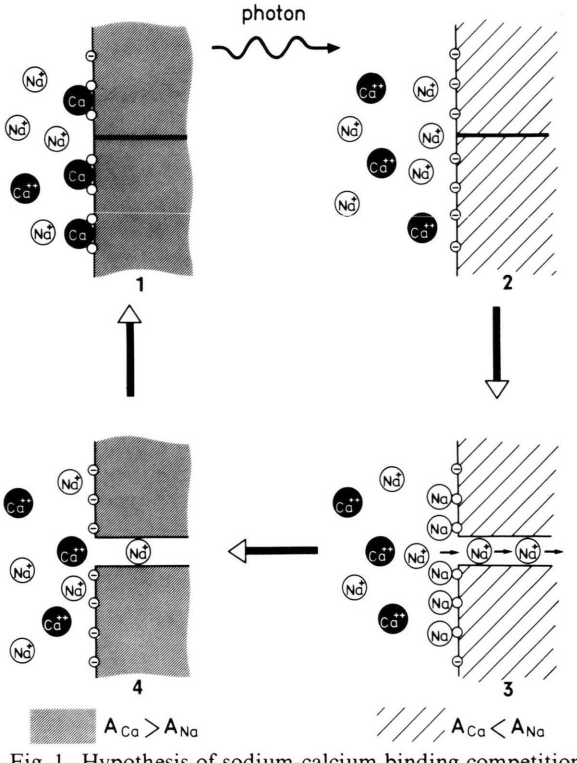

Fig. 1. Hypothesis of sodium-calcium binding competition of negatively charged binding sites which control the gating of light-activated ion channels in the Limulus photosensory membrane. Light channel is closed when calcium is bound, and opened when sodium is bound instead. A_{Ca}: affinity for calcium, A_{Na}: affinity for sodium. (1) Strong affinity for calcium in the dark, channel closed. (2) Photon absorption induces transient affinity change, raising the relative affinity for sodium; calcium and sodium ions compete for binding sites. (3) Under normal conditions the extracellular sodium concentration is much higher than that of calcium, so that sodium is bound and the channel opens. (4) The affinity changes spontaneously back in favour of calcium; under normal conditions calcium is bound (1) and the channel closes again (from [18]).

Materials and Methods

Ventral photoreceptor nerves were excised from adult *Limuli*, freed from the blood-vessel and kept in physiological saline. Before the experiments the nerves were bathed for 45 s in physiological saline containing 0.5% pronase. For details of preparation and procedure see [1, 19]. The preparations were fixed on a sylgard bedding in a plexiglass test vessel, which was permanently perfused throughout the experiment with a flow rate of ca. 1 ml/min. The half-time of the exchange of salines was ca. 1 min at the preparation. In the test salines with reduced sodium concentration the osmotic pressure was kept constant by replacing the sodium ions by choline or lithium ions. In the reference salines for the test salines with increased Ca^{2+}-concentration sucrose was added for the same reason. (For details see [1].) The pH of all salines was close to 7.5. The ionic composition of the salines used is shwon in Table I.

Stimulating light, measuring and recording system

The preparation was stimulated from below through the bedding by white light from a xenon lamp (VIX 150) administered through a light pipe. The intensity of the stimulating light could be attenuated by neutral density filters (Schott), the maximal intensity was ca. 2×10^5 lx, equivalent to 6×10^{15} (550 nm) photons $cm^{-2}\,s^{-1}$. Glass microelectrodes filled with 0.5 mol/l KCl were inserted into the photoreceptor cell under microscope ($100 \times$ magnification). The resistance of the sensor electrode (measuring the membrane voltage) was between 10 and 15 MΩ, that of the driver electrode (for current injection) was between 5 and 10 MΩ. Ag/AgCl electrodes were used. The indifferent electrode was placed in the surrounding saline. The recording circuit used to measure the unclamped membrane voltage and the membrane current under voltage clamp conditions is shown in Fig. 2. The gain of the feedback amplifier was 1000, the frequency range from dc to 500 Hz (3 dB).

The data were digitized and recorded on tape, photographed from a dual beam oscilloscope. The time resolution of the recording system was 1 ms, the accuracy of the voltage measurements 1 mV, that of the current measurements 0.5 nA.

Procedure

In the experiments light stimuli of maximal intensity available (evoking saturated voltage response amplitude) were administered. The stimulus duration was 10 ms (in one group of experiments 2 s) throughout the experiment. One measurement episode lasted 10 s; the time of one measuring cycle was 60 s. After successful impalement of the cell with two microelectrodes each experiment started with a pre-period of at least 15 minutes without background illumination, in which the cell was allowed to adapt to the experimental conditions. During the pre-period, the test vessel was perfused by physiological saline. After the receptor potentials evoked by the identical stimuli had become constant, the cell was voltage clamped with a clamp regime consisting of several steps (Fig. 3). First the cell was hyperpolarized to −100 mV for 2 seconds and than the clamp voltage jumped to the desired test voltage U_{Cl} (for 6 s) which was varied from

Table I. Composition of salines [mmol/l].

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	lithium	choline	EDTA	HEPES	glucose	PO_4^{3-}	Saccharose
1 a (PS)	483	10	10	55	563	30	–	–	–	10	–	–	–
b	48	10	$< 10^{-3}$	–	565	–	517	–	1	10	–	–	–
c	483	10	$< 10^{-3}$	–	565	–	82	–	1	10	–	–	–
2 a (PS)	483	10	10	55	563	30	–	–	–	10	–	–	–
b	48	10	$< 10^{-3}$	–	565	–	–	517	1	10	–	–	–
c	483	10	$< 10^{-3}$	–	565	–	–	82	–	10	–	–	–
3 a (PS)	477	10	10	55	557	30	–	–	–	10	10	0.1	–
b	48	10	10	–	575	–	498	–	–	10	10	0.1	–
4 a (PS)	481	10	10	55	561	30	–	–	–	10	–	–	90
b	481	10	40	55	621	30	–	–	–	10	–	–	–
5 a (PS)	435	10	10	55	515	30	–	–	–	10	–	–	270
b	435	10	100	55	695	30	–	–	–	10	–	–	–

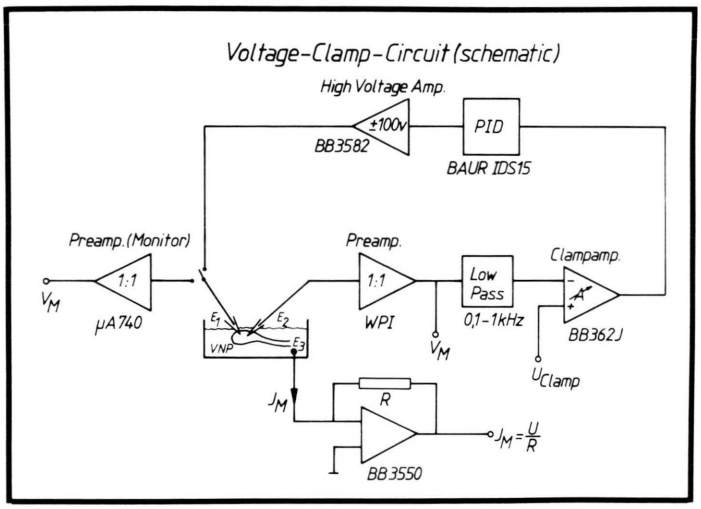

Fig. 2. Scheme of the voltage clamp circuit used. Ventral nerve photoreceptor (VNP), voltage electrode (E_1), current electrode (E_2), indifferent electrode (E_3), membrane current (J_M), resistance (R), current voltage converter (BB 3550), preamplifier (WPI), membrane voltage (V_M), low pass filter (0.1 – 1 kHz), clamp amplifier (BB 362J), proportional integral differential control (PID, BAUR JDS15), high voltage amplifier (BB 3582), preamplifier (monitor).

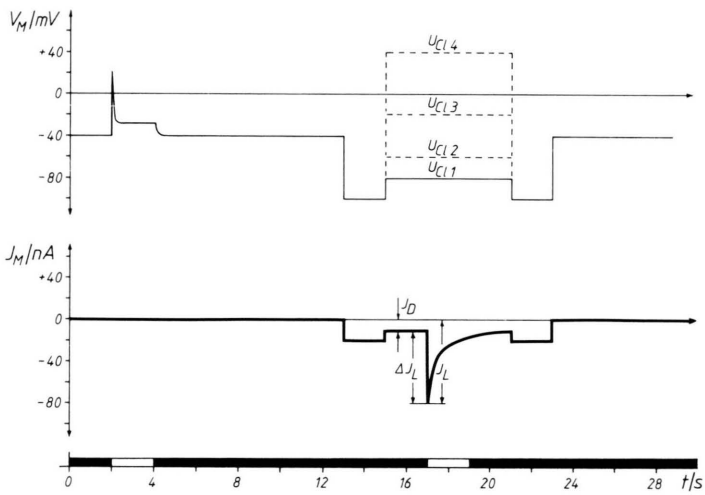

Fig. 3. Episode of measurement; upper trace: scheme of membrane voltage (U_{Cl}); lower trace: membrane current including demonstration of measured parameters: dark current (J_D), amplitude of light-induced current (ΔJ_L), total light current (J_L).

cycle to cycle, then the voltage jumped back to −100 mV for 2 seconds, and finally the clamp circuit was switched off again. The hyperpolarizing voltage of −100 mV was chosen to make the measurements of the different cycles comparable, since hyperpolarization to this degree has turned out to be favourable for the function of voltage sensitive ionic channels in the plasma membrane of excitable cells. By variation of the clamp voltage from cycle to cycle, starting from hyperpolarization to −100 mV up to depolarization to +45 mV, dark currents J_D, total light currents J_L and light induced currents ΔJ_L could be measured at membrane voltages clamped to the different values. Before and after each clamp episode receptor potentials of the unclamped cell were recorded to check the condition of the preparation.

After a first period in physiological saline, the perfusion of the test chamber was switched to the first test saline (second period). Constant stimuli were applied to the unclamped cell until responses became stable again, and then again the membrane currents were measured for different clamp voltages. If a second test saline was used measurements were made in the same way. The most stressing saline (low $[Ca^{2+}]_{ex}$ together with normal $[Na^+]_{ex}$) was ap-

plied as last test saline, to keep the preparation in good condition as long as possible. Finally, during the after-period, the ventral nerve was again superfused by physiological saline and the regime of measurements was performed as in the other periods. The experiments lasted ca. 1.5 hours following successful penetration of the cell. The duration of the periods was between 15 and 30 min, depending on the time until stable responses were obtained. The temperature was constant, between 15 and 20 °C in different experiments.

Evaluation

From the unclamped cell the pre-stimulus membrane potential (PMP), and the peak amplitude (HMAX) of the receptor potential were measured. When the cell was voltage-clamped the membrane current during the whole clamp episode was recorded. From this recording the membrane current in the dark (J_D), the amplitude of its change due the light stimulus (the light induced membrane current ΔJ_L), and the corresponding total light current ($J_L = J_D + \Delta J_L$) were measured (Fig. 3). In each experiment the curves of the measured membrane current data *vs.* the respective clamp voltage were plotted, and their reversal potentials determined. For a closer description of the parameters see the list of abbreviations.

Definition of parameters

The reversal potential of the photoreceptor membrane in the dark, $V_{rev}J_D$, is determined by the ion specifities of the ion channels open in the dark ("dark channels"), and the concentration gradients of the respective ions. The reversal potential of a sound photoreceptor cell of *Limulus* in the dark is 40–50 mV (equal to the value of the pre-stimulus membrane potential). The membrane conductance in the dark (g_D) is determined by the product of number and single channel conductance of the dark channels, plus the leakage conductance. The reversal potential of the light-induced current, $V_{rev}\Delta J_L$, is determined by the ion specifities of the light-activated channels, and the concentration gradients of the ions passing through these "light channels". (The reversal potential of the light-induced current does not depend on the number of light-activated channels. The light-induced current is in first approximation proportional to the number of light-activated channels. The reversal potential $V_{rev}J_L$ of the total light current, J_L, is always between that of the dark current, J_D, and the light-induced current, ΔJ_L (Fig. 4): $V_{rev}J_D < V_{rev}J_L < V_{rev}\Delta J_L$. The value of $V_{rev}J_L$ depends upon the ratio of the contribution of the dark conductance and the light-induced conductance of the photosensory membrane.)

The light-induced membrane conductance, Δg_L, is the product of number and single channel conductance of the light-activated channels. The total membrane conductance during illumination, g_L, for a given membrane voltage, is the sum of dark and light-induced membrane conductances. (This does not apply to the data in Table II, since these membrane conductances were determined (as "slope conductances") at the respective reversal potentials, *i.e.* at different voltages for dark, light-induced, and total light membrane conductance.) The pre-stimulus membrane potential, PMP, and the reversal potential in the dark, $V_{rev}J_D$, should be identical under normal conditions. This is the case for most of our results. The maximal response amplitude, HMAX, after a saturating light stimulus, was recorded between the clamp episodes to check the condition of the preparation. For stimuli evoking a saturated response amplitude HMAX should be equal to the difference between $V_{rev}J_D$ and $V_{rev}J_L$, if the contribution of the voltage-sensitive conductances to the peak amplitude of the (unclamped) receptor potential can be neglected.

Results and Interpretation

The current voltage relation was measured over a voltage clamp range from −100 to +45 mV first in physiological saline and than in different test salines. The average results of the experiments are presented in Table II. The experiments differ by the sodium-substitute; while lithium was used in the first and third group, choline was taken in the second group. Since in two groups of experiments (1 and 2) the calcium concentration of the test salines was lowered, the third group was made with lowered sodium concentration but normal calcium concentration. In two further groups of experiments (not presented in Table II) the effect of increased calcium concentration was tested.

The actual concentrations of calcium and sodium at the outer surface of the photosensory membrane

are probably somewhat attenuated as compared to the values of Table I, because the partially digested glia cell layer acts as diffusion barrier.

Effect of low calcium concentration and low or normal sodium concentration (sodium substitute: lithium) on the light response

In the first group of experiments (Table II, 1 a – c, Figs. 4 and 5) the preparations were exposed to PS (a), test saline with low $[Ca^{2+}]_{ex}$ ($< 10^{-9}$ mol/l) and low $[Na^+]_{ex}$ (10% of normal value, Na^+-substitute: lithium) (b) and test saline with low $[Ca^{2+}]_{ex}$ and normal $[Na^+]_{ex}$ (c). Figure 4 shows recordings of the membrane voltage (unclamped) and the membrane current (clamp voltage indicated at each measurement) of one experiment of this group. Membrane current *vs.* voltage curves of the same experiment are shown in Fig. 5.

When the external concentration of divalent cations is very low ($< 10^{-6}$ mol/l by omitting Ca^{2+} and Mg^{2+} and using EDTA as chelating agent) and the $[Na^+]_{ex}$ is additionally lowered (Table II, 1 b; Fig. 5) the prestimulus membrane potential PMP is shifted to slightly positive values and the amplitude of the receptor potential (HMAX) is markedly reduced or even abolished. The light-induced conductance increase and the corresponding membrane current have become smaller. The reversal potential of the dark current is changed to positive values, while the reversal potential of the light-induced current and that of the total light current are slightly reduced in the saline with low calcium and sodium concentrations, as can be expected due to the decreased sodium gradient.

Under these low calcium, low sodium conditions the membrane conductance in the dark g_D is increased (Table II, 1 b) and the light-induced con-

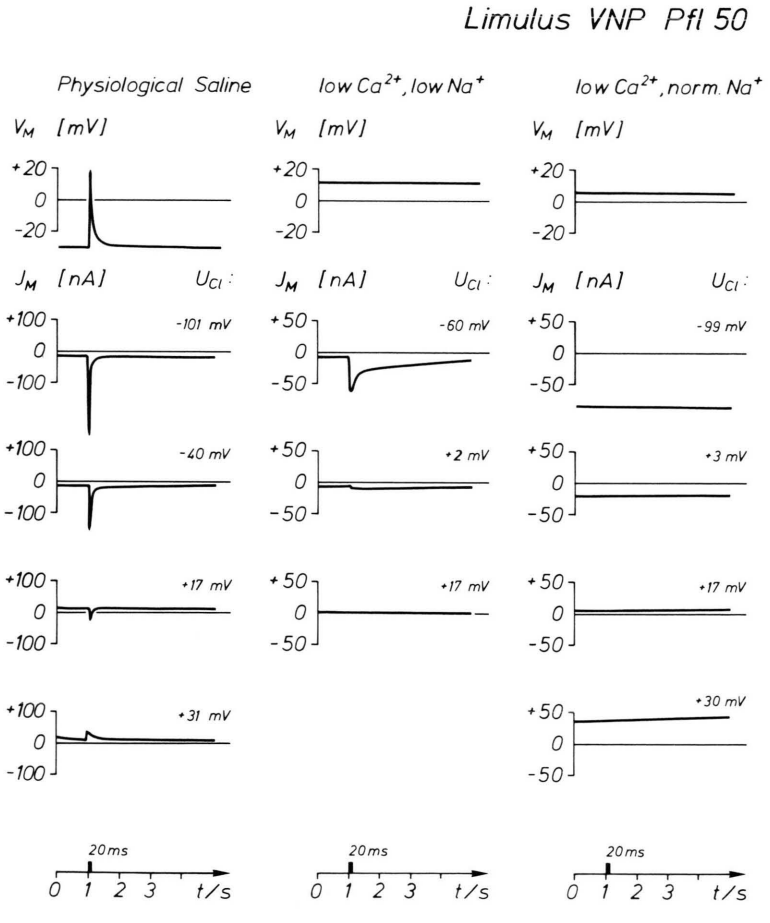

Fig. 4. Receptor potentials (upper row) and receptor currents (lower rows) of *Limulus* ventral nerve photoreceptor, recorded in PS (first column), low calcium, low sodium test saline (second column), and low calcium, normal sodium test saline (third column). Sodium substitute: lithium. For composition of salines see Table I, salines 1 a – c. 20 ms white light stimuli, intensity equivalent to 6×10^{15} (550 nm) photons $cm^{-2}\,s^{-1}$. Clamp voltage as indicated. Temperature: 15 °C. PFL 50.

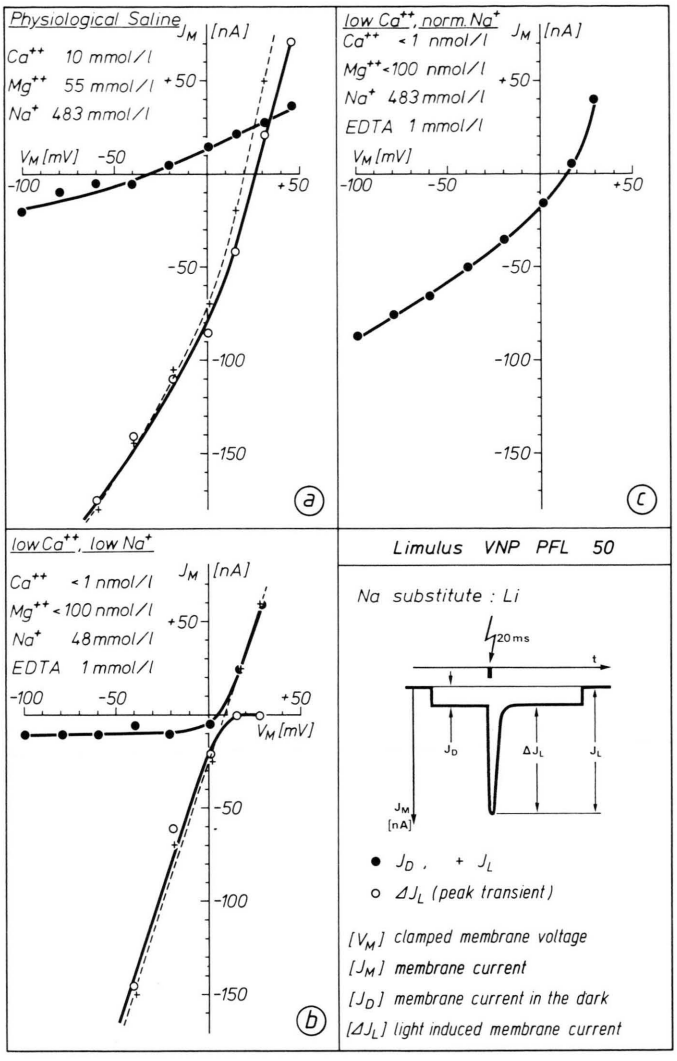

Fig. 5. Membrane current *vs.* clamp voltage measurements in *Limulus* ventral nerve photoreceptor in PS and test salines as indicated. Measured parameters shown by inset. Same experiment as Fig. 4, see there for details. Values of reversal potentials in PS (a) $V_{rev}J_D$ − 30 mV, $V_{rev}J_L$ + 20 mV, $V_{rev}\Delta J_L$ + 25.5 mV; in low calcium, low sodium saline (b) $V_{rev}J_D$ + 6 mV, $V_{rev}J_L$ + 9 mV, $V_{rev}\Delta J_L$ + 17 mV; in low calcium, normal sodium saline (c) $V_{rev}J_D$, $V_{rev}J_L$, $V_{rev}\Delta J_L$ + 15 mV. Values of slope conductances in PS (a) g_D 0.4 μS; g_L 4.4 μS, Δg_L 4.2 μS; in low calcium, low sodium saline (b) g_D 1.4 μS, g_L 3.2 μS, Δg_L 2.8 μS; in low calcium, normal sodium saline (c) g_D, g_L 1.5 μS, Δg_L 0 μS. Values of pre-stimulus membrane potentials (last record in each saline) in PS (a) − 30 mV, in low calcium, low sodium saline (c) + 6 mV, in low calcium, normal sodium saline (c) + 9 mV. Values of HMAX (last record in each saline) in PS (a) 50 mV, in low calcium, low sodium saline (b) 9 mV, in low calcium, normal sodium saline (c) 0 mV. PFL 50.

ductance (Δg_L) is somewhat reduced as compared to PS. This could be expected according to our hypothesis, provided that the ion specifity of dark and light channels is not (much) influenced by the test saline. (Since the membrane conductances are measured at the respective reversal potentials the sum of g_D and Δg_L in Table II has not to be equal to g_L). Raising of the $[Na^+]_{ex}$ to its physiological value while the $[Ca^{2+}]$ is still low (Table II, 1 c) leads to a positive value of the PMP (+ 20 mV), close to the value of the reversal potential of the total light current ($V_{rev}J_L$) in PS. The light response is reduced to zero. Consequently dark current and total light current are equal. The same applies to dark conductance and total light conductance, and the corresponding reversal potentials (Fig. 5). These results could be expected from our working hypothesis. The reversal potential of the dark current in this calcium-deficient saline is even slightly (but not significantly) more positive (+ 24 mV) than $V_{rev}J_L$ in PS (+ 20 mV) and close to $V_{rev}\Delta J_L$ in PS (+ 25 mV), Table II, 1 a, c.

According to our working hypothesis these results can be interpreted in the following way: The fact that the dark conductance is increased in a calcium-deficient saline with normal $[Na^+]_{ex}$ may be due either to an increase of the number of open ion channels, or to increased single channel conductance.

Table II. Average values (± standard error of the mean) for all groups of experiments. For experimental details see figure legends. Deviating numbers of n are indicated. For salines see Table I. Pre-stimulus membrane potential PMP and receptor potential amplitude HMAX, both measured as last values in each saline; reversal potential of dark current, ($V_{rev}J_D$) reversal potential of light current ($V_{rev}J_L$); reversal potential of light-induced current ($V_{rev}\Delta J_L$); membrane conductance in the dark (g_D); total membrane conductance during illumination, (g_L); light-induced membrane conductance (Δg_L); conductances measured at zero membrane currents, i.e. when reversal potentials are reached; dark current (J_D), total light current (J_L), and maximum of light induced current (ΔJ_L), at clamp voltage − 50 mV. PFL 42, 45, 46, 50 (1); PFL 25, 30, 31, 32 (2); PFL 85, 86, 87 (3).

		1 ($n=4$)				2 ($n=4$)				3 ($n=3$)			
		a PS	b low Ca low Na lithium	c low Ca normal Na		a PS	b low Ca low Na choline	c low Ca normal Na		a PS	b normal Ca low Na lithium	c PS	
PMP	[mV]	32 ± 2.5	2 ± 3.3	20 ± 9.1(3)	− 23 ± 2.3		− 19 ± 5.8	7 ± 2.2		− 36 ± 3.9	− 37 ± 2.6	− 40 ± 4.5	
HMAX	[mV]	50 ± 2.3(3)	13 ± 5.2(3)	0	31 ± 4.8		24 ± 2.6	2.1 ± 0.8		51 ± 3.2	29 ± 3.2	47 ± 1.5	
$V_{rev}J_D$	[mV]	− 32 ± 2.3	7 ± 7.0	24 ± 7.5(3)	− 23 ± 2.6		− 9 ± 9.6	8 ± 1.7		− 36 ± 2.2	− 36 ± 2.7	− 33 ± 7.5	
$V_{rev}J_L$	[mV]	20 ± 3.6	16 ± 4.4(3)	24 ± 7.5(3)	11 ± 5.7		11 ± 5.2	14 ± 4.5		18 ± 2.7	− 6 ± 8.3	10 ± 3.7	
$V_{rev}\Delta J_L$	[mV]	26 ± 0.9	20 ± 4.5(2)	0	27 ± 6.2		17 ± 4.9	16 ± 6.1		21 ± 2.6	12 ± 4.0	16 ± 2.8	
g_D	[μS]	0.6 ± 0.2	1.8 ± 0.7(3)	1.4 ± 0.1(2)	0.4 ± 0.04		0.5 ± 0.08	5 ± 1.2		0.4 ± 0.06	0.3 ± 0.03	0.3 ± 0.04	
g_L	[μS]	4 ± 1.0(3)	5 ± 1.4(2)	1.4 ± 0.1(2)	1.5 ± 0.6		5.5 ± 0.7	9.7 ± 0.6		6.9 ± 0.8	1.4 ± 0.5	4 ± 1.0	
Δg_L	[μS]	3.8 ± 0.9(3)	3.2 ± 0.4(2)	0	1.6 ± 0.8		4.1 ± 0.5	4 ± 1.5		6.5 ± 0.8	0.6 ± 0.27	3.8 ± 0.9	
J_{D-50}	[nA]	− 10 ± 5.1	− 57 ± 31	− 102 ± 46$_2$	− 7 ± 1.3		− 15 ± 2.8	− 91 ± 27		− 5.1 ± 0.97	− 2.8 ± 0.1	− 3.8 ± 1.4	
J_{L-50}	[nA]	124 ± 34	− 363 ± 182	− 102 ± 46$_2$	− 56 ± 25		− 74 ± 6.9	− 211 ± 12		429 ± 22	− 98 ± 53	− 201 ± 69	
ΔJ_{L-50}	[nA]	− 104 ± 35	− 351 ± 268	0	− 49 ± 25		− 59 ± 6.0	− 120 ± 22		− 424 ± 21	− 95 ± 52	− 197 ± 68	

A further effect of calcium deficiency – the shifting of the reversal potential of the dark current (or the PMP) towards the value of the reversal potential of the total light current – could be caused by an increasing contribution of the normally light-activated channels, opening already in the dark under low calcium conditions, additionally to the contribution of the dark channels. This assumption explains also the third effect of calcium deficiency, the reduction or abolition of the light-induced conductance increase. Lowering of the sodium concentration counteracts both the shifting of the reversal potential of the dark current and the reduction of light-induced conductance increase.

The fact that the reversal potential of the light-induced current slightly decreases in a calcium-deficient saline might indicate that a small fraction of the light-induced current is carried by calcium ions (compare [20]). The sum of the membrane conductance in the dark and the light-induced conductance change (*i.e.* the total light conductance g_L) should not depend on the $[Ca^{2+}]_{ex}$, provided that the number of functional dark channels and the number of light channels activated by the light stimulus, and the conductances of the single channels are constant. (This should apply *e.g.* for the current measurement at the clamp voltage of − 50 mV.) The increase of the membrane conductance in the dark, or the light current at a clamp voltage of − 50 mV observed in our experiments could be caused by "sleeping channels" activated by calcium deficiency. Another explanation would be that low calcium conditions increase the single channels conductance. The latter should probably be accompanied by a decrease in the ion specifity. However such a decrease in ion specifity can be ruled out since the reversal potential of the light-induced current stays at about + 15 mV to + 25 mV in low calcium saline as long as it is measurable.

Effect of low calcium concentration and low or normal sodium concentration (sodium substitute: choline) on the light response

The effect of lowering the $[Na^+]_{ex}$ obviously depends on the sodium substitute. If choline, instead of lithium, is used as sodium substitute (second group of experiments, Table II, 2a–c, Figs. 6 and 7) the changes are generally similar, but the effect of lowering the $[Na^+]_{ex}$ is more pronounced

with choline than with lithium. With choline as substitute the sodium reduction leads to a quantitatively better compensation of the effect caused by the lowering of the divalent cations; that is to say, lithium is more similar to sodium than choline.

(In this group of experiments stimuli of 2 s duration were applied as compared to the 10 ms stimuli used in the other experiments. Within these limits the stimulus duration has no marked influence on the results.) When sodium is reduced and replaced by choline, together with reduced $[Ca^{2+}]_{ex}$ (Table II, 2b) the changes as compared to PS consist in only a moderate reduction of the amplitude of the ReP (from 30 to 25 mV) and only a slight depolarization (from −23 to −19 mV) of the PMP. The reversal potential of the dark current is shifted to a less negative value (from −23 to −9 mV) and the reversal potential of the total light current is almost the same as in PS. The dark conductance is slightly increased, while the light conductance is increased from 1.5 to 5.5 µS. In low calcium, low sodium saline the reversal potential of the light-induced current is decreased (from 27 to 17 mV). The plot of the light-induced membrane current *vs.* clamp voltage (Fig. 7b) shows a section with a negative slope, which is caused by voltage dependence of the light-activated channels.

Under low calcium, normal sodium conditions (Table II, 2c) the effects are about the same as observed in the corresponding test saline with lithium as sodium substitute. The effects should be similar, depending only on the influence of the 80 mmol/l lithium or choline, by which the sodium gradient is practically unchanged compared to PS, and on the conditions of the different preparations. The persistence of the light response in low calcium, normal sodium saline (Table II, 2c) suggests that in this saline stationary values had not been reached. After longer stay in test saline the light response disappears. So there is still a light-induced membrane current, which would be expected to disappear under normal sodium, low calcium conditions, *i.e.* part of the channels can still be activated by light.

The reversal potential of the dark current is shifted to a positive value (from −9 to +8 mV) in the low calcium, normal sodium saline, while $V_{rev}\Delta J_L$ and $V_{rev}J_L$ remain practically the same as in

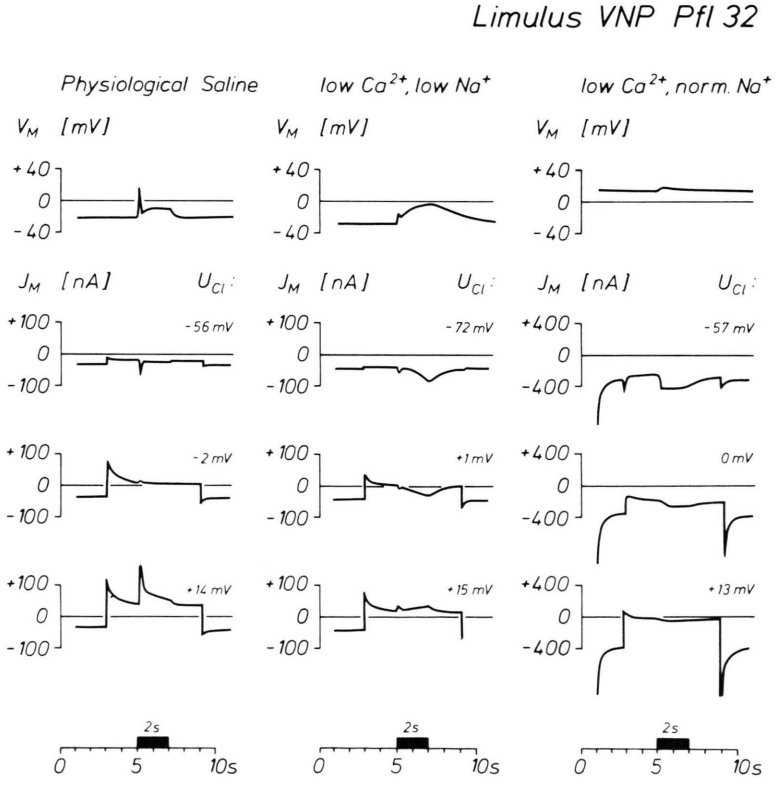

Fig. 6. Receptor potential (upper row) and receptor currents (lower rows) of *Limulus* ventral nerve photoreceptor recorded in PS (first column), low calcium, low sodium test saline (second column) and low calcium, normal sodium test saline (third column). Sodium substitute: choline, for salines see Table I, salines 2a–c. 2 s white light stimuli, intensity equivalent to 6×10^{15} (550 nm) photons $cm^{-2}\,s^{-1}$. Clamp voltage as indicated. Temperature 15 °C. PFL 32.

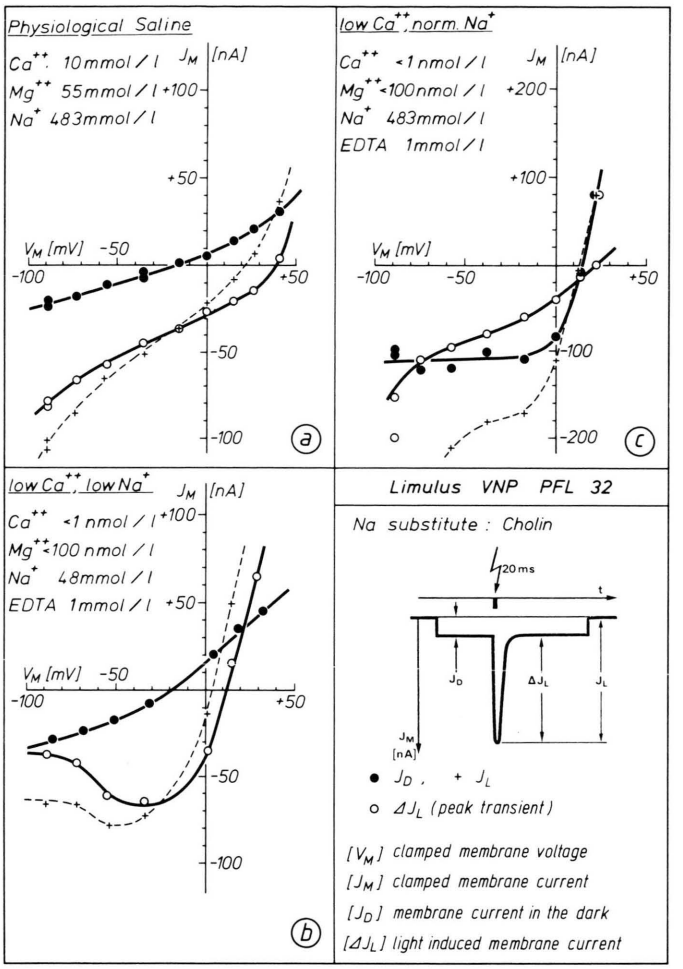

Fig. 7. Membrane current *vs.* clamp voltage measurements in *Limulus* ventral nerve photoreceptor in PS and test salines as indicated. Measured parameters shown by inset. Same experiment as in Fig. 6, see there for details. Values of reversal potentials in physiological saline (a) $V_{rev}\,J_D$ − 14 mV, $V_{rev}\,J_L$ + 22 mV, $V_{rev}\,\Delta J_L$ + 35 mV; in low calcium, low sodium saline (b) $V_{rev}\,J_D$ − 19 mV, $V_{rev}\,J_L$ + 4 mV, $V_{rev}\,\Delta J_L$ + 12 mV; in low calcium, normal sodium saline (c) $V_{rev}\,J_D$ + 14 mV, $V_{rev}\,J_L$ + 13 mV, $V_{rev}\,\Delta J_L$ + 22 mV. Values of slope conductances in PS (a) g_D 0.4 μS, g_L 1.3 μS, Δg_L 0.6 μS; in low calcium, low sodium saline (b) g_D 0.7 μS, g_L 7.1 μS, Δg_L 3.5 μS, in low calcium, normal sodium saline (c) g_D 6.7 μS, g_L 9.1 μS, Δg_L 2.1 μS. Value of the PMP (last record in each saline) in PS (a) − 20 mV; in low calcium, low sodium saline (b) − 21 mV; in low calcium, normal sodium saline (c) + 12 mV. Values of HMAX (last record in each saline) in PS (a) 34 mV; in low calcium, low sodium saline (b) 24 mV; in low calcium, normal sodium saline (c) 2 mV. PFL 32.

low calcium, low sodium saline (Fig. 7). The dark conductance is much increased (from 0.5 to 4.5 μS) (Table II, 2c). Dark und light currents at a clamp voltage of − 50 mV become much larger than in the preceding low calcium, low sodium saline. The average light-induced outward current at a clamp voltage + 25 mV in the low calcium, low sodium saline was 6 nA for lithium and 56 nA for choline as sodium substitute. At the same clamp voltage the light-induced outward current in a normal calcium, low sodium saline (with lithium, see below) was 2 nA, *i.e.* still lower than when the $[Ca^{2+}]_{ex}$ was reduced too. This indicates that (1) lithium passes through the light channels less easily than sodium and (2) the outward current in a low sodium saline is even more reduced than the inward current for lithium as sodium substitute. In some experiments with low sodium saline (and lithium) there was no light-induced outward current, *i.e.* no current reversal, for positive clamp voltages, no matter whether the $[Ca^{2+}]_{ex}$ was lowered or normal.

Effect of normal calcium concentration and low sodium concentration (sodium substitute: lithium) on the light response

The effect of lowering the $[Na^+]_{ex}$ alone to 10% of its normal value in a saline containing normal concentrations of divalent cations was tested in the third group of experiments (Table II, columns 3a−c, Figs. 8 and 9). Lowering the sodium concentration does not change the PMP and the reversal potential of the dark current (Table II, 3b). How-

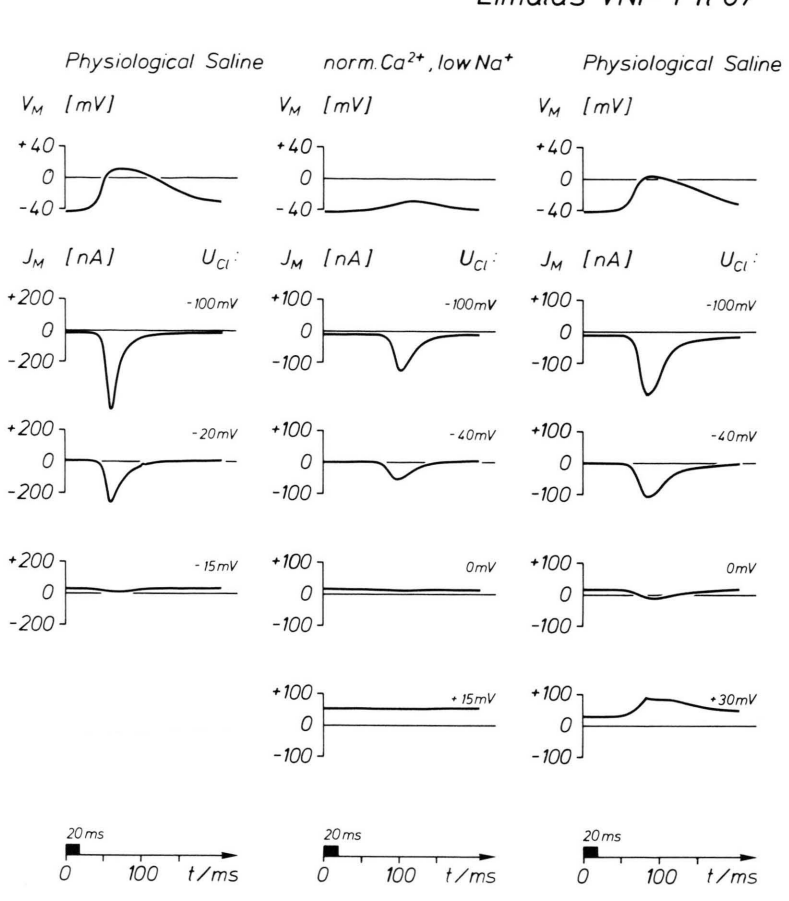

Fig. 8. Receptor potentials (upper row) and receptor currents (lower rows) of *Limulus* ventral nerve photoreceptor recorded in PS (first column), low sodium, normal calcium saline (second column), and again in PS (third column). Sodium substitute: lithium. For salines see Table I, salines 3a, b. Further details as described for Fig. 4. Temperature 15 °C, PFL 87.

ever the amplitude of the light response (HMAX) is reduced almost by half, and the reversal potential of the light-induced current is lowered from +20 to +10 mV (Fig. 9b). The dark conductance is slightly reduced (from 0.4 to 0.3 μS) while the light-induced conductance is lowered from 6.5 to 0.6 μS. At a clamp voltage of −50 mV the dark current is reduced by half while the light-induced current is much more diminished (from 430 to 100 nA) in the sodium-deficient saline. The effects are well reversed upon return to physiological saline (Fig. 9c). The decrease of $V_{rev}\Delta J_L$ can be explained by the reduction of the sodium gradient. The decrease of dark and light membrane conductances may be due to a smaller contribution of sodium ions passing through the light-activated channels, together with a smaller contribution of the dark channels.

Effect of raised calcium concentration and normal sodium concentration of the light response

The effect of raising the $[Ca^{2+}]_{ex}$ to 40 mmol/l and 100 mmol/l on the current/voltage relation was tested in two groups of experiments (Fig. 10).

In contrast to the effect of reducing the $[Ca^{2+}]_{ex}$, raising of the $[Ca^{2+}]_{ex}$ to 40 mmol/l has no significant effect on the current/voltage relation. Raising the $[Ca^{2+}]_{ex}$ to 100 mmol/l leads to hyperpolarization of the dark potential, a negative (hyperpolarizing) shift of the reversal potential in the dark $V_{rev}J_D$, and a positive shift of the reversal potential of the light induced current $V_{rev}\Delta J_L$ (Fig. 10). The reversal potential of the total light current is practically unchanged, the same applies to the slope conductances.

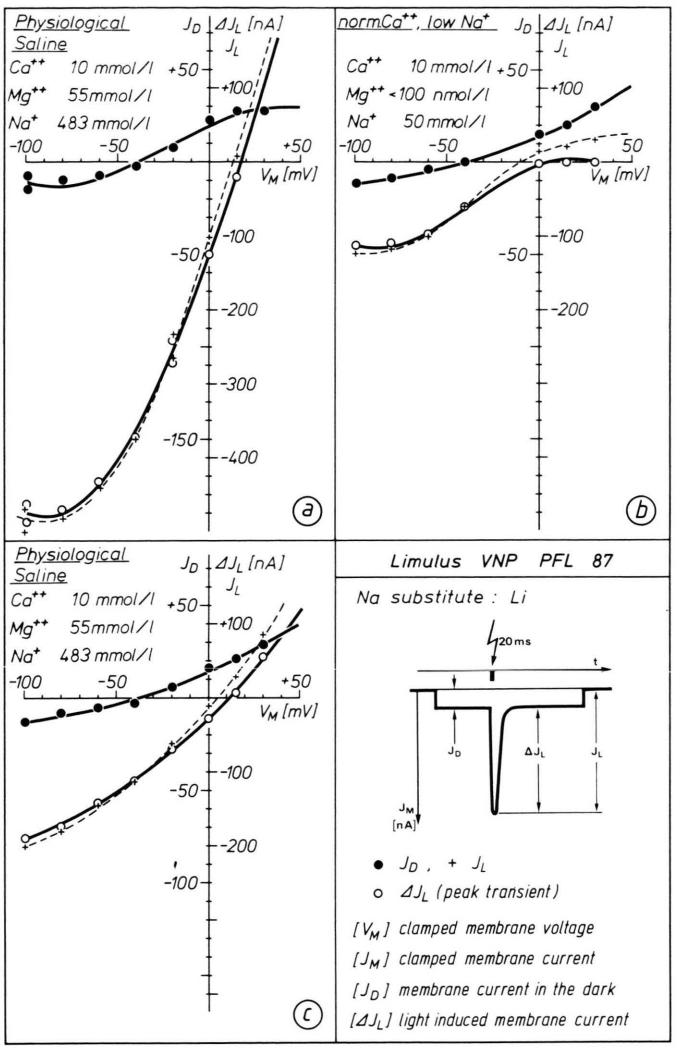

Fig. 9. Membrane current *vs.* clamp voltage measurements in *Limulus* ventral nerve photoreceptor in PS, low sodium test saline, and again PS as indicated. Measured parameters shown by inset. Same experiments as in Fig. 8, see there for details. Values of reversal potentials in physiological saline (a) $V_{rev}J_D$ − 38.5 mV, $V_{rev}J_L$ + 14 mV, $V_{rev}\Delta J_L$ + 17 mV; in normal calcium, low sodium saline (b) $V_{rev}J_D$ − 40 mV, $V_{rev}J_L$ − 10 mV, $V_{rev}\Delta J_L$ + 4 mV; again in PS (c) $V_{rev}J_D$ − 40 mV, $V_{rev}J_L$ + 4.5 mV, $V_{rev}\Delta J_L$ + 11 mV. Values of slope conductances in Ps (a) g_D 0.5 µS, g_L 8.5 µS, Δg_L 8.0 µS; in normal calcium, low sodium saline (b) g_D 0.3 µS, g_L 1.6 µS, Δg_L 0.7 µS; again in PS (c) g_D 0.3 µS, g_L 3.0 µS, Δg_L 2.6 µS. Values of the PMP (last record in each saline) in PS (a) − 40 mV, in normal calcium, low sodium saline (b) − 41 mV, again in PS (c) − 41 mV. Values of HMAX (last record in each saline) in PS (a) 51 mV, in normal calcium, low sodium saline (b) 26 mV, again in PS (c) 44 mV, PFL 87.

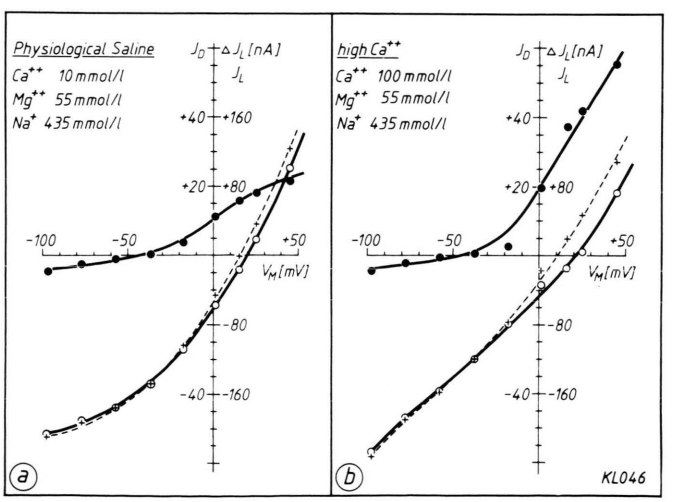

Fig. 10. Membrane currents *vs.* clamp voltage measurements in *Limulus* ventral nerve photoreceptor in PS and in a test saline in which the calcium concentration was raised to 100 mmol/l. For salines see Table I, salines 5 a, b. Experimental details and parameters as in Figs. 4, 8. Values of reversal potentials in PS (a) $V_{rev}J_D$ − 44 mV, $V_{rev}J_L$ 16 mV, $V_{rev}\Delta J_L$ 20 mV, and in high calcium saline (b) $V_{rev}J_D$ − 41 mV, $V_{rev}J_L$ 11 mV, $V_{rev}\Delta J_L$ 20 mV. Values of slope conductances in PS (a) g_D 0.13 µS, g_L 3.2 µS, Δg_L 2.6 µS, and in high calcium saline (b) g_D 0.15 µS, g_L 2.9 µS, Δg_L 1.6 µS. Values of the PMP (last record in each saline) in PS − 47 mV, and in high calcium saline (b) − 48 mV. Values of HMAX (last record in each saline) in PS (a) 59 mV, and in high calcium saline (b) 48 mV. KLO 46.

High $[Ca^{2+}]_{ex}$ causes no greater changes of the membrane characteristics of the photoreceptor cell membrane. This means that even with an external calcium concentration as high as 100 mmol/l the light-activated channels are still functioning. The effects of the high calcium saline are most plausibly explained by assuming that, by the raised calcium concentration, the ion selectivity of both dark and light-activated channels is not changed. The leakage conductance may be somewhat reduced. The light-induced conductance increase is smaller, either due to a reduction of the number of light-activated channels per photon absorbed, or (and) to a decrease of the single channel conductance. The reversal potential of the total light current, which is determined by the balance of dark and light-activated channels, weighted by channel number and single channel conductance, is practically not changed by raising the $[Ca^{2+}]_{ex}$. The increase of the pre-stimulus membrane potential and the reversal potential of the light current in the saline with increased $[Ca^{2+}]_{ex}$ may be due to less leakage, so that the ion specifity of the channels becomes dominating.

Whereas the gating process in the visual cell membrane is strongly influenced by calcium deficiency, the effect of increased calcium concentration is not significant.

Conclusions

The results are in general agreement with the predictions from our model. The assumption that low calcium conditions cause an unspecific increase in the membrane conductance is ruled out by our results since the pre-stimulus membrane potential and the reversal potential of the dark current become positive instead of approaching zero as would be the case for an unspecific conductance change.

We think it rather improbable that extremely low calcium concentration of the external saline strongly alters the ion specifity of either the dark channels or the light-activated channels. However we cannot rule out that the single channel conductance and/or the number of functional channels is increased in low calcium saline. The effect of lithium as sodium substitute can be compared with that of magnesium as substitute for calcium: both compete for the same binding sites with the substance they replace; but with a weaker action. Lithium is a bad current carrier. Magnesium cannot (or only very weakly) replace calcium in the process of adaptation [21]. In our experiments the antagonistic effect of lowering the $[Na^+]_{ex}$ is smaller when lithium is used as sodium substitute than when choline is used. Lithium is more similar to sodium than choline.

Under very low calcium conditions the light activated ion channels in the visual cell membrane open already in the dark. The total membrane conductance (dark conductance plus light-induced membrane conductance) increase in a calcium-deficient saline, especially when choline is used as substitute for sodium. Our results, which agree with the findings cited in the introduction, can be explained by our working hypothesis (Fig. 1): The gating of light-activated ion channels is regulated by the calcium concentration. When calcium is bound to the controlling binding sites in the cell membrane the channels are closed; when the bound calcium is replaced by sodium, the channels open. In the dark the controlling binding sites have a strong affinity for calcium ions. The light stimulus causes a transient reduction in the calcium affinity of the binding sites which allows sodium to replace the bound calcium. Sodium can be bound already in the dark when the $[Ca^{2+}]_{ex}$ is drastically reduced. A comparatively small additional reduction in the $[Na^+]_{ex}$ restores the light-activated gating process almost to normal, since the ratio $[Ca^{2+}]/[Na^+]$ is improved again to above a critical value. A further increase (beyond the critical value) of this ratio has no influence on the gating mechanism.

The increase of the dark and light membrane conductance under low calcium conditions is an additional observation not part of our channel gating hypothesis.

Acknowledgements

We thank R. Schanzer for technical help. This work was supported by the DFG, SFB 160.

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