Extracellular Calcium, Magnesium, and Sodium Ion Competition in the Conductance Control of the Photosensory Membrane of *Limulus* Ventral Nerve Photoreceptor

H. Stieve and M. Bruns

Institut für Neurobiologie der KFA Jülich

Z. Naturforsch. **33 c**, 574–579 (1978); received May 3, 1978

Limulus Photoreceptor, Membrane Conductance, Calcium/Sodium Competition

The membrane potential in the dark and the saturated response height of the ventral nerve photoreceptor of *Limulus* was measured by an intracellular electrode while the external concentration of calcium, magnesium and sodium ions was varied.

Decreasing the extracellular calcium concentration from 10^{-2} mol/l causes a calcium-dependent lowering of the dark membrane potential and at very low concentrations ($< 10^{-8}$ mol/l a reversal to ca. +5 to +11 mV, if the external magnesium concentration is also low. Also, the light response diminishes with decreasing extracellular calcium concentration and disappears at a concentration of 10^{-9} mol/l.

External magnesium can substitute for certain properties of extracellular calcium.

Lowering the extracellular sodium concentration from 543 mmol/l to 30–50 mmol/l reduces the dark membrane potential and the light responses at normal calcium concentration, whereas at low calcium concentration it causes a substantial rise of both.

Interpretation: The results are in accordance with our working hypothesis that a strong reduction of the external calcium (and magnesium) concentration causes a calcium concentration dependent opening of "light channels" in the dark. Additional lowering the extracellular sodium concentration counteracts this effect; opening and closing of light channels is controlled by negative binding sites on the cell membrane for which calcium and sodium ions compete with an antagonistic action.

Introduction

The receptor potential of the arthropod visual cell is caused by a transient light-induced conductance increase of the photosensory membrane. One can assume an underlying mechanism in which light causes a temporary opening of ion channels ("light channels"), in the photosensory membrane, whereas permanently open "dark channels" are responsible for the steady membrane potential in the dark (summary of ref. [1]).

The effect of lowering the external calcium, magnesium and sodium concentration on arthropod photoreceptors has been studied by several authors:

Reduction of $[Ca^{2+}]_{ex}$ * to concentrations $\geqq 10^{-5}$ mol/l (in the presence of Mg^{2+}) generally causes no substantial decrease in the membrane potential in the dark but a significant increase in the amplitude of the light response [2–8]. The light-induced membrane current becomes larger, the reversal voltage of the light-induced current is not changed [2, 6, 9].

Omitting the magnesium alone from the bathing solution of *Limulus* ventral nerve photoreceptor caused a 10% increase of the light response both for receptor potential and the light-induced membrane current, without affecting the membrane potential in the dark or the reversal potential of the light-induced membrane current [9, 10]. The light response of the hermit crab and *Limulus* is abolished when calcium and magnesium are effectively removed by EDTA [7, 11–13].

Brown and Blinks [10] also observed, in *Limulus* ventral nerve photoreceptor, that in the absence of calcium and magnesium in the presence of 5 mmol/l EGTA, within 1–2 min the membrane voltage becomes positive in the dark and approaches (and sometimes becomes more positive than) the reversal voltage for the electrical response to light. With the cell in this condition, they found still a large light-induced change in membrane conductance as measured with a voltage clamp.

Lowering the external sodium concentration is reported on the one hand, not to influence the membrane potential in the dark [2, 5, 6] on the other, to decrease it somewhat [14]. The light response becomes somewhat smaller [3, 7, 15], and the reversal potential of the light-induced current is reported to be somewhat decreased [2, 6, 9].

Requests for reprints should be sent to Prof. Dr. H. Stieve, Institut für Neurobiologie der KFA Jülich GmbH, Postfach 19 13, D-5170 Jülich 1.

* Extracellular calcium concentration; from now on symbol $[\ ]_{ex}$ is used to denote extracellular ion concentrations.

Based on experiments with retinular cells of *Limulus* (lateral eye) and *Astacus* [12, 13, 16] we proposed a hypothesis that the amount of calcium bound to the external surface of the photosensory membrane determines whether the light channels are open or closed. Lowering the external concentration of calcium ions decreases the membrane dark potential and the maximal response height of the receptor potential; it increases the membrane dark conductance and makes the light-induced conductance increase smaller. The effect of lowering the external calcium concentration can be counteracted by additionally reducing the external sodium concentration [13]. This antagonistic action suggests a calcium/sodium competition for binding sites at the photosensory membrane.

An antagonism of calcium and sodium in arthropod photoreceptors has been observed also by other authors: Brown *et al.* [2] report a Ca/Na antagonism in the suppression of the light-induced permeability increase of the *Balanus* photoreceptor, which they interpret as a binding competition of Ca and Na for a presumptive Na-carrier or carrier site complex; a model with many similarities to ours. Brown and Ottoson [17] also report a competition between Ca^{2+} and K^+ ions in the conductivity control of the photosensoric membrane of *Balanus*.

A different type of sodium/calcium interaction is reported by Lisman and Brown [4]. Intracellular sodium injection causes a progressive transient diminution of the sensitivity of *Limulus* ventral nerve photoreceptor which depends on the extracellular calcium ion concentration: the higher $[Ca^{2+}]_{ex}$, the higher the desensitization. When $[Ca^{2+}]_{ex}$ was 0.1 mmol/l, iontophoretic Na^+-injection caused negligible progressive decrease in the size of the light response. However, the desensitizing action of intracellular injection of Ca^{2+} still leads to the diminution of the light response in 0.1 mmol/l $[Ca^{2+}]_{ex}$.

Using a wider range of calcium and magnesium concentrations than in our former experiments we investigated further the antagonistic action of calcium and sodium on conductance control in the ventral nerve photoreceptor of *Limulus*.

Experimental Procedure

Test salines with different calcium, sodium and magnesium concentrations were used. The membrane potential and receptor potential of the ventral nerve photoreceptor of *Limulus* were measured intracellularly while the experimental chamber containing the ventral nerve was continuously perfused with salines of various calcium and sodium concentrations. The flow rate of the solution was 1 ml/min. When the perfusion of the chamber was switched to another saline, the half time of the exchange at the preparation was ca. 1 min. Throughout the experiment the ventral nerve was stimulated every 60 s by a 20 ms flash of response-saturating intensity. Each experiment consisted of five periods as shown in Fig. 1:

a) 30 min in physiological saline;
b) 10 min in a saline with low $[Ca^{2+}]_{ex}$ and low $[Na^+]_{ex}$;
c) 15 min in a saline with low $[Ca^{2+}]_{ex}$, and normal $[Na^+]_{ex}$;
d) 15 min in the same saline as in period b;
e) 120 min in physiological saline as in period a.

The omitted calcium, magnesium, or sodium ions were replaced by choline ions to keep the osmotic pressure constant. For further details of methods and procesure see table legends.

Results

Fig. 1 shows the course of a typical experiment. The changes in membrane potential and receptor potential caused by a simultaneous reduction of $[Ca^{2+}]_{ex}$ and $[Na^+]_{ex}$ (b-period) develop slowly and are not complete in this particular case during the b-period. However, increasing $[Na^+]_{ex}$ while maintaining low $[Ca^{2+}]_{ex}$ (c-period) induces a faster change and an almost stationary value is reached within 5 min. The rapidity of the response is even more pronounced in the d-period when $[Na^+]_{ex}$ is reduced for a second time. These fast changes are especially relevant to our interpretation. Finally, return to physiological saline from low $[Ca^{2+}]_{ex}$ salines often causes a transient anesthesia-like effect and it normally takes considerable time for the preparation to recover.

Table I shows the results of 4 groups of experiments with different external calcium concentrations $[Ca^{2+}]_{ex}$. The values were obtained from the last stimulation of the respective period, at which time (with the exception of 5, upper line) the parameters had reached almost steady values. It is seen that upon decreasing $[Ca^{2+}]_{ex}$ the membrane potential

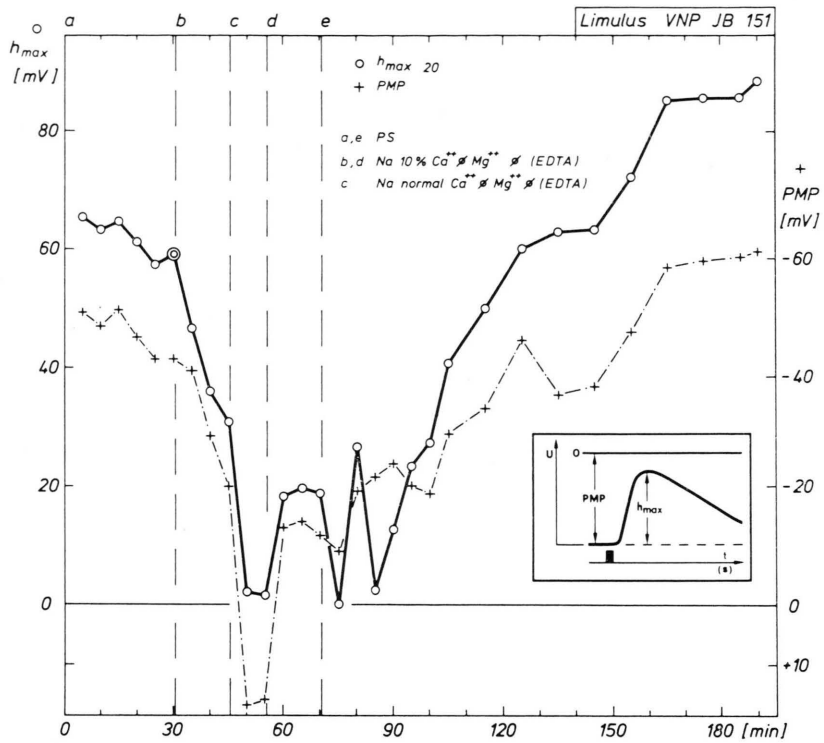

Fig. 1. Course of membrane potential PMP and saturated response amplitude h_{max} upon 20 ms white light stimulus (2×10^5 lx, corresponding to 6×10^{15} photons·cm^{-2}·s^{-1} of 550 nm) during stay in physiological saline (P.S. $[Ca^{2+}] = 10^{-2}$ mol/l) in periods a and e, low sodium (10%) and low calcium ($<10^{-9}$ mol/l) saline in periods b and d, and normal sodium and low calcium ($<10^{-9}$ mol/l) saline in period c. This is one of the experiments listed in Table I, line 5, see there for composition of salines. Every 5th response plotted. *Limulus* ventral nerve photoreceptor.

Table I. Membrane potential PMP and saturated light response amplitude h_{max} (average values and S.E., normalized to the average of the reference values listed in upper line 1) depending on extracellular calcium and sodium concentration. Experiments JB 142–155 *Limulus* ventral nerve photoreceptor. 20 ms light stimulus, white light from Xenon lamp, intensity 2×10^5 lx, corresponding to 6×10^{15} photons·cm^{-2}·s^{-1} of 550 nm. Temperature 15 °C.
Line 1, upper values: averages of PMP and h_{max} of a-periods (physiological saline, composition as shown, plus 10^{-2} mol/l K^+, 6.08×10^{-1} mol/l Cl^-, 10^{-2} mol/l HEPES; pH 7.5).
Line 1, lower values: averages of PMP and h_{max} of a-periods in physiological saline with decreased sodium content, values in parantheses are from different experiments (JB 85–88 *Limulus* lateral eye).
Line 2 to 5: values of PMP and h_{max} in different test salines as shown. Left values of PMP and h_{max} columns were measured at normal sodium concentration (period c), right values at low sodium concentration (averages of periods b and d). When calcium, magnesium, or sodium ions were omitted, they were replaced by choline ions to keep the osmotic pressure constant. The lower $[Ca^{2+}]_{ex}$ and $[Mg^{2+}]_{ex}$ were buffered by EGTA or EDTA. Tris was not used as pH buffer because it may interfere with EGTA [20].
The concentration of free calcium ions was determined according to Portzehl *et al.* [21].

	$[Ca^{2+}]_{ex}$ [mol/l]	$[Mg^{2+}]_{ex}$ [mol/l]	Complexing agent [mol/l]	$[Na^+]_{ex}$ [mol/l]	n	PMP [mV]		h_{max} [mV]	
1	10^{-2}	5.5×10^{-2}	—	5.4×10^{-1}	15	**-45 ± 2.7**		**58 ± 2.3**	
				1.4×10^{-5}	(4)		(-32 ± 5.4)		(35 ± 3.4)
2	5×10^{-5}	5×10^{-3}	—	5.4×10^{-1}	3	-13 ± 5.4		22 ± 7.5	
				38.4×10^{-3}	6		-22 ± 2.6		22 ± 5.3
3	10^{-6}	5×10^{-3}	2×10^{-3} EGTA	5.4×10^{-1}	5	-11 ± 6.8		22 ± 2.4	
				5.4×10^{-3}	10		-21 ± 4.2		23 ± 5.4
4	5×10^{-5}	5×10^{-5}	—	5.4×10^{-1}	4	$+4\pm1.5$		9 ± 4.3	
				38.4×10^{-3}	8		-20 ± 3.2		12 ± 4.0
5	$<10^{-9}$	$<10^{-7}$	10^{-3} EDTA	4.8×10^{-1}	2	$+9\pm5.4$		2 ± 1.0	
				48×10^{-3}	4		-15 ± 3.0		19 ± 4.4

decreases and reverses in sign at calcium concentrations below 5×10^{-5} mol/l, if the $[Mg^{2+}]_{ex}$ is also very small.

The effect of lowering the $[Na^+]_{ex}$ depends upon the $[Ca^{2+}]_{ex}$: In normal $[Ca^{2+}]_{ex}$ the dark membrane potential is decreased by lowering the $[Na^+]_{ex}$ (lower line 1), whereas the decreased membrane potential in low $[Ca^{2+}]_{ex}$ is partially restored (becomes more negative) due to lowering the $[Na^+]_{ex}$ (compare upper and lower lines 2 to 5). Even the reversed values of the membrane potential of +4 and +9 mV in normal $[Na^+]_{ex}$ (upper lines 4 and 5) are restored to more normal negative values by lowering the $[Na^+]_{ex}$ (lower lines 4 and 5).

With decreasing $[Ca^{2+}]_{ex}$ and $[Mg^{2+}]_{ex}$ in normal $[Na^+]_{ex}$ saline, the saturated response height decreases gradually and becomes almost zero in $[Ca^{2+}]_{ex} < 10^{-9}$ mol/l (see also Fig. 1). Upon a prolonged stay in this saline (30 min), the light response is abolished. We chose the shorter length (15 min) of the c-period in this saline in order to cause as little injury to the preparation as possible. If the $[Na^+]_{ex}$ is additionally lowered, the response recovers even under these low $[Ca^{2+}]_{ex}$ conditions. With normal $[Ca^{2+}]_{ex}$, however, lowering the $[Na^+]_{ex}$ gives a reduction in the response height.

The values of the membrane potential and the saturated response height do not differ much between lines 2 and 3 despite the fact that the $[Ca^{2+}]_{ex}$ is changed by a factor of 50, from 5×10^{-5} to 10^{-6} mol/l. Obviously at this low calcium concentration the effect of magnesium is dominating and stabilizes the membrane potential and response height at the values given. Going to a lower magnesium concentration (5×10^{-5} mol/l, line 4) consequently results in great changes of membrane potential and response height.

The stabilizing effect of external magnesium at low calcium concentrations was tested in a separate set of experiments (Table II).

Table II, line 2 shows normal values of the membrane potential (-47 ± 4.6 mV) and maximal amplitude of light response (65 ± 4.9 mV) in physiological saline. Lowering the $[Mg^{2+}]_{ex}$ to 5×10^{-5} mol/l in a saline containing normal calcium and sodium concentration does not cause a substantial diminution of the membrane potential or of the maximal amplitude of the light response (line 1). Decreasing $[Ca^{2+}]_{ex}$ to 10^{-9} mol/l and $[Mg^{2+}]_{ex}$ to 5×10^{-5} mol/l reduces the membrane potential to +0.5 mV and the light response height to 9 mV (line 5). If the $[Mg^{2+}]_{ex}$ is subsequently raised to 10^{-1} mol/l (line 6), the membrane potential is restored to more than half its reference value (-27 mV) in physiological saline and the response height is also restored to almost half its reference value (31 mV). Lines 3 and 4 show a similar somewhat smaller effect at higher $[Ca^{2+}]_{ex}$ and lower $[Mg^{2+}]_{ex}$.

These experiments show that the effect of magnesium on membrane potential and response height is similar to, but weaker than, that of calcium. Therefore the $[Mg^{2+}]_{ex}$ was also lowered when the effect of very low $[Ca^{2+}]_{ex}$ was studied.

Table II. Membrane potential PMP and saturated light response amplitude h_{max} (average values and S.E., normalized to the average of the refercence values listed in line 2) depending on extracellular calcium and magnesium concentration. Experiments JB 128–130, 135–139, 146–148, 152–155, *Limulus* ventral nerve photoreceptor. Further details as in Table I.

Line 2: averages of PMP and h_{max} of a-periods (physiological saline as in Table I).

Lines 1, and 3 to 6: values of PMP and h_{max} in different test salines as shown. Left values of PMP and h_{max} columns measured at low magnesium (periods c), right values at increased magnesium concentrations (periods b and d). Both left and right values at equal calcium concentration. Approximately normal sodium concentration in all experiments.

	$[Ca^{2+}]_{ex}$ [mol/l]	$[Mg^{2+}]_{ex}$ [mol/l]	Complexing agent [mol/l]	$[Na^+]_{ex}$ [mol/l]	n	PMP [mV]	h_{max} [mV]
1	10^{-2}	5×10^{-5}	—	5.5×10^{-1}	3	-45 ± 4.2	64 ± 2.3
2	10^{-2}	5.5×10^{-2}	—	5.4×10^{-1}	15	**-47 ± 4.6**	**65 ± 4.9**
3	5×10^{-5}	5×10^{-5}	—	5.4×10^{-1}	4	$+4 \pm 1.6$	10 ± 4.8
4	5×10^{-5}	5×10^{-3}	—	5.4×10^{-1}	3	-14 ± 5.6	24 ± 8.5
5	10^{-9}	5×10^{-5}	10^{-3} EGTA	5.7×10^{-1}	5	$+0.5 \pm 2.2$	9 ± 3.8
6	$< 5 \times 10^{-9}$	10^{-1}	10^{-3} EGTA	4.2×10^{-1}	9	-27 ± 5.6	31 ± 8.5

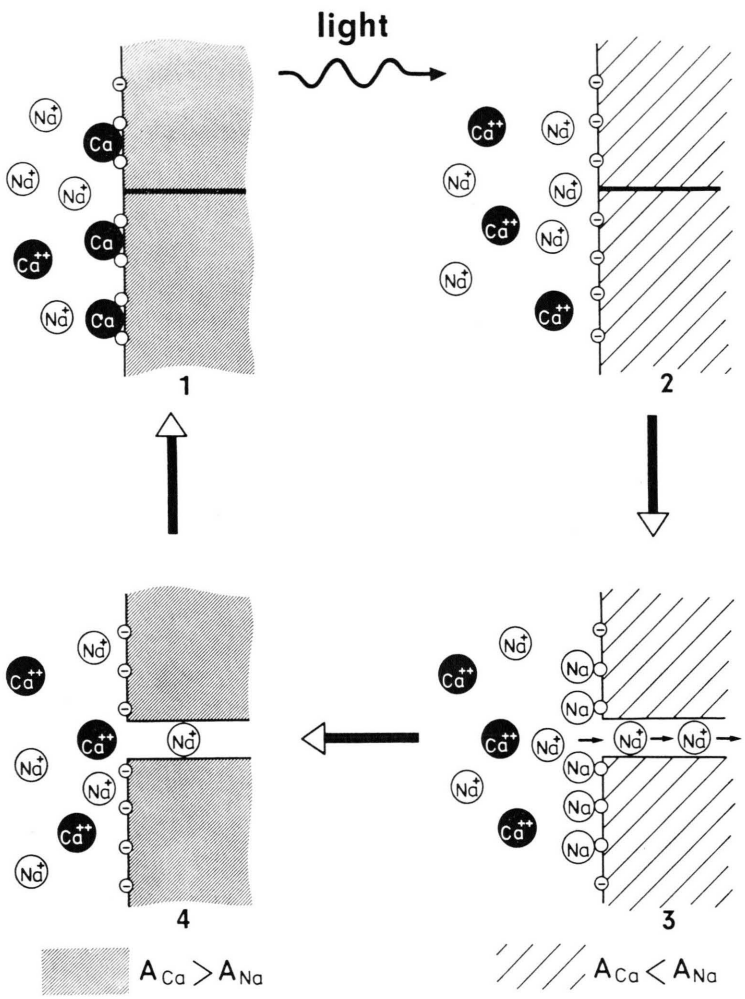

Fig. 2. Hypothesis of sodium-calcium binding competition for negatively charged binding sites controlling opening and closing of light activated ion channels in the arthropode photosensory membrane. Light channel is closed when calcium is bound, and opened when sodium is bound instead.
A_{Ca}: affinity for calcium, A_{Na}: affinity for sodium.
(1) Strong affinity for calcium in the dark, channel closed.
(2) Photon absorption causes transient affinity change, raising the relative affinity for sodium; calcium and sodium 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 back spontaneously in favour of calcium; under normal conditions calcium is bound and the channel closes again (1) (from ref. [22]).

Discussion

The observed facts are consistent with the hypothesis that calcium and sodium ions compete for negatively charged binding sites at the photosensory cell membrane. The binding sites control the membrane conductance and especially the activation of the light channels: the less calcium and consequently, the more sodium, is bound, the more light channels are (permanently) open already in the dark. Voltage clamp experiments which were performed under similar conditions [18] confirm this hypothesis:

The same test salines as in the group of experiments listed in Table I, line 5 were applied in the same sequence in a group of voltage clamp experiments to determine the current-voltage-relation (I-V-curve) of the *Limulus* ventral nerve photoreceptor using two intracellular electrodes. Lowering the $[Ca^{2+}]_{ex}$ to $<10^{-9}$ mol/l and $[Mg^{2+}]_{ex}$ to $<10^{-7}$ mol/l in normal $[Na^{+}]_{ex}$ causes a ca. 25-fold increase in the membrane dark conductance (as measured by the slope of the I-V-curve at reversal potential). The reversal potential of the membrane dark current is changed to $+7$ mV. This is close to the value of the total membrane light current which is not changed by calcium removal.

Simultaneous decrease of $[Ca^{2+}]_{ex}$, $[Mg^{2+}]_{ex}$ and $[Na^{+}]_{ex}$ causes only a 2-fold increase of the membrane dark conductance as compared to the value in physiological saline. The reversal potentials of dark current and of light-induced current are both only slightly decreased by $5-10$ mV.

Lowering the divalent cation concentration shifts the following three potentials to nearly the same voltage as the maximum of the receptor potential:

a) the membrane potential in the dark (its sign is reversed), b) the reversal potential of the membrane dark current, c) the reversal potential of the total membrane light current. These observations are in accord with an increase of the membrane conductivity of the same ion selectivity as that of the light channels; they contradict an unspecific leakiness of the photosensory membrane.

Because of the reduced divalent ion concentration the dark membrane conductance is strongly increased while the light-induced conductance increase is greatly diminished. This favours the assumption that actually the light channels are opened by Ca^{2+}-depletion, over the alternative that an additional channel type is created, parallel to the light channels with the same ion selectivity.

The fact that magnesium ions have a weaker effect than calcium ions leads to the conclusion that calcium exerts its influence by binding to the cell membrane rather than by screening.

The effect of external calcium and sodium concentration on the activation of light channels can be explained in two possible ways:

(a) Permanent fixation of a certain adjusted amount of calcium to the membrane surface is a necessary pre-requisite for the function (opening and closing) of the light channels. The amount of fixed calcium depends on the external calcium, magnesium, sodium and, according to Brown and Ottoson also potassium concentration. When too few sites are occupied by calcium (and the majority by sodium) the light channels should be open even in the dark.

(b) A transient release of calcium, which has been bound to the external membrane surface and which can be replaced by sodium ions, is a causal step for the activation of the light channels. For instance binding of calcium could cause the light channels to close, whereas a replacement of the bound calcium by binding of sodium could cause opening. A hypothetical scheme of such a sodium-calcium binding competition at the photosensory membrane is shown in Fig. 2. The light stimulus could cause a transient affinity change of the negative binding sites, reducing transiently the preference for calcium over sodium ions.

This second possibility of our model has (except for the antagonistic action of sodium) several features in common with the model proposed by Weeks and Duncan [19].

Our results are in good agreement with the findings of Brown *et al.* [2] in the barnacle photoreceptor. Aside from the striking similarities between our two models there is a main difference: Our assumption, that light channels are opened in the dark by lowering the external concentration of the divalent ions. This, however, is derived from observations in much lower divalent ions concentrations than those used by them.

We thank G. Smith, K. Hartung, E. Hildebrand, and J. Pink for discussion of the results, and H. Gaube, E. Brentgens, and B. Stieve for help with the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 160).

[1] H. Stieve, Biophys. Struct. Mechanism **3,** 145–151 (1977 a).
[2] H. M. Brown, S. Hagiwara, H. Koike, and R. M. Meech, J. Physiol. **208,** 385–413 (1970).
[3] B. Fulpius and F. Baumann, J. Gen. Physiol. **53,** 541–561 (1969).
[4] J. E. Lisman and J. E. Brown, J. Gen. Physiol. **59,** 701–719 (1972).
[5] R. Millecchia and A. Mauro, J. Gen. Physiol. **54,** 310–330 (1969 a).
[6] R. Millecchia and A. Mauro, J. Gen. Physiol. **54,** 331–351 (1969 b).
[7] H. Stieve, Z. vergl. Physiol. **47,** 457–492 (1964).
[8] H. Stieve Verh. Dtsch. Zool. Ges. **1974,** 19–23 (1975).
[9] J. E. Brown and M. I. Mote, J. Gen. Physiol. **54,** 331–351 (1974).
[10] J. E. Brown and J. R. Blinks, J. Gen. Physiol. **64,** 643–665 (1974).
[11] H. Stieve, Cold Spring Harbor Symp. Quant. Biol. **XXX,** 451–456 (1965).
[12] H. Stieve, Biochemistry and Physiology of Visual Pigments (H. Langer, ed.), 237–244, Springer-Verlag, Berlin, Heidelberg, New York 1973.
[13] H. Stieve, Biochemistry of Sensory Functions (L. Jaenicke, ed.), 79–105, Springer-Verlag, Berlin, Heidelberg, New York 1974.
[14] V. J. Wulff, Vision Res. **13,** 2309–2326 (1973).
[15] R. B. Clark and G. Duncan, Biophys. Struct. Mechanism (1978).
[16] H. Stieve, Bioelectrochem. Bioenerg. **3,** 151–157 (1976).
[17] H. M. Brown and D. Ottoson, J. Physiol. **257,** 355–378 (1976).
[18] H. Stieve and M. Pflaum, Vision Res. (1978), in press.
[19] F. I. Weeks and G. Duncan, Exp. Eye Res. **19,** 483–509 (1974).
[20] Y. Ogawa, J. Biochem. **64,** 255–257 (1968).
[21] H. Portzehl, P. C. Caldwell, and J. C. Rüegg, Biochim. Biophys. Acta **79,** 581–591 (1964).
[22] H. Stieve, Biophysik – Ein Lehrbuch (W. Hoppe *et al.*, eds.), p. 482–501, Springer-Verlag, Berlin, Heidelberg 1977 b).