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# Calcium is necessary for light excitation in barnacle photoreceptors

Uri Werner, Edith Suss-Toby, Ayelet Rom, and Baruch Minke*

Department of Physiology and the Minerva Center for Studies of Visual Transduction
The Hebrew University-Hadassah Medical School, Jerusalem, 91010 Israel

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**Summary.** Illumination of barnacle *(Balanus amphitrite)* photoreceptors is known to increase the membrane permeability to sodium and $Ca^{2+}$ ions resulting in a depolarizing receptor potential. In this report, we show that lanthanum ($La^{3+}$), a known inhibitor of Ca-binding proteins, reversibly eliminates the receptor potential of barnacle photoreceptors when applied to the extracellular space. Similar reversible elimination of the light response was obtained by removing extracellular $Ca^{2+}$ by application of the calcium chelating agent EGTA. Iontophoretic injection of $Ca^{2+}$, but not $K^+$ into the cells protected both the transient and the steady-state phases of the receptor potential from elimination by EGTA while only the transient phase was protected in the presence of $La^{3+}$. The EGTA experiments suggest that internal $Ca^{2+}$ is *necessary* for light excitation of barnacle photoreceptors while the $La^{3+}$ experiments suggest that $La^{3+}$-sensitive inward current is necessary to maintain excitation during prolonged light.

**Key words:** *Balanus* – Lanthanum – EGTA – trp mutant

## Introduction

Illumination of the barnacle lateral ocelli results in a conductance increase primarily to sodium ions, leading to a depolarizing receptor potential with a reversal potential of about +25 mV (Brown et al. 1970; review Meech and Brown 1976). Illumination of the barnacle photoreceptors leads also to an increase in intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) as detected by aequorin luminescence (Brown and Blinks 1974), by arsenazo III and by $Ca^{2+}$ selective microelectrodes (Brown et al. 1988). The aequorin luminescence can be abolished by replacing extracellular $Ca^{2+}$ ($[Ca^{2+}]_o$) with sucrose suggesting that the increase in $[Ca^{2+}]_i$ arises from an influx of $Ca^{2+}$ from the extracellular space (Brown and Blinks 1974). In the absence of external $Na^+$ an increase in $[Ca^{2+}]_o$ shifts the reversal potential of the light response indicating that the light-sensitive channels are permeable to $Ca^{2+}$ (Brown et al. 1970).

A large body of evidence strongly suggests that $Ca^{2+}$ is an intracellular messenger of light adaptation in invertebrate photoreceptors (Brown et al. 1970; Lisman and Brown 1972; Brown and Lisman 1975; Brown and Blinks 1974; reviews: Brown 1986; Payne et al. 1988). It has been proposed that $Ca^{2+}$ also participates in excitation in *Limulus* ventral photoreceptors (Stieve and Bruns 1980; Bolsover and Brown 1985; Payne et al. 1986; Frank and Fein 1991) and in the barnacle lateral ocelli (Brown et al. 1988). In *Limulus* ventral photoreceptors it has been found that: i) most of the increase in cellular $Ca^{2+}$ arises from a release from intracellular pools (Brown and Blinks 1974) which makes the control of internal $Ca^{2+}$ difficult. ii) Calcium buffers (EGTA and BAPTA), which abolish exogenous inositol trisphosphate ($InsP_3$) – and $Ca^{2+}$-induced excitation do not abolish, but only reduce and slow down, the light-induced excitation (Payne et al. 1986; Frank and Fein 1991). The barnacle photoreceptor provides a useful preparation to examine the role of $Ca^{2+}$ in excitation since the increase in $[Ca^{2+}]_i$ arises mainly from an influx of $Ca^{2+}$ from the extracellular space, and thus can be easily controlled. Therefore, we examined the effect of inhibiting $Ca^{2+}$ influx into barnacle photoreceptor on the receptor potential. Two methods which are likely to inhibit $Ca^{2+}$ influx were used: i) reduction in $[Ca^{2+}]_o$ by low $[Ca^{2+}]_o$ combined with EGTA and ii) blocking $Ca^{2+}$ entry by extracellular lanthanum ($[La^{3+}]_o$).

In fly photoreceptors there is a sustained response to prolonged light. Application of $La^{3+}$ extracellularly to the photoreceptors suppresses the sustained component of the light response resulting in a transient response to prolonged intense light. The concentration of $La^{3+}$ used

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*Abbreviations:* *EGTA* ethylenglyol-bis-(β-aminoethylether) N, N, $N^1$, $N^1$-tetraacetate; *BAPTA* bis-(0-aminophenoxy)-ethane-N, N, $N^1$, $N^1$-tetraacetic acid; *DMSO* dimethyl sulfoxide; *trp* transient receptor potential; *nss* no steady state; *ASW* artificial sea water

* To whom offprint requests should be sent

in the fly was in the range of 1–100 μ*M* in a perfused retina and 5 m*M* when applied in a small drop into the intact fly eye. The transient response in the presence of $La^{3+}$ recovers within 1 min in the dark (Hochstrate 1989; Suss-Toby et al. 1991). A very similar transient light response to prolonged intense light is observed in the *t*ransient *r*eceptor *p*otential (*trp*) mutant of *Drosophila* (Cosens and Manning 1969; Minke et al. 1975; Minke 1982) and in the *no steady state* (*nss*) mutant of the sheep blowfly *Lucilia* (Howard 1982, 1984; Barash et al. 1988; Suss et al. 1989). In *Limulus* ventral and photoreceptors, on the other hand, extracellular $La^{3+}$ of 100 μ*M* (in 1 m*M* $Ca^{2+}$-ASW) or 1 m*M* (in 10 m*M* $Ca^{2+}$-ASW) has little if any effect on the light response (Stieve et al. 1976; Z.-X. Zhang and A. Fein; J.E. Brown, personal communication). These diverse actions of $La^{3+}$ on the photoreceptors of different invertebrate species, together with the reported difference between the *Limulus* and the barnacle regarding $Ca^{2+}$ influx into the photoreceptors (Brown and Blinks 1974), suggest that the detailed mechanism of phototransduction is somewhat different in the *Limulus* relative to the other invertebrate species such as the fly and the barnacle. Therefore, it would be interesting to examine the effect of $La^{3+}$ on the barnacle photoreceptors.

## Materials and methods

*The preparation.* Barnacles *(Balanus amphitrite)* were obtained from Haifa, Israel. The lateral ocelli were excised with a short section of nerve and placed in a perfusion chamber. Pronase (1.5%, Calbiochem) and collagenase (1.5%, Sigma) were applied for 4 min. A transparent connective tissue which covers the photoreceptors was removed by a pipette following the enzymatic treatment and the ocellus was fixed in the perfusion chamber with petroleum jelly.

*Chemicals and solutions.* The dissected eye was perfused by ASW containing in m*M*: 423 NaCl, 10 KCl, 10 $CaCl_2$, 22.1 $MgCl_2$, 26.2 $MgSO_4$, 17.2 Hepes buffer, pH 7.4. After about 10 min of perfusion, the normal ASW was usually replaced by 0.1 m*M* Ca-ASW in which the $CaCl_2$ concentration was reduced to 0.1 m*M* $CaCl_2$ and 30.4 m*M* sucrose was added to keep the osmolarity constant. In some experiments the dissected eye was perfused by 0.1 m*M* Ca-ASW only. When EGTA (7.5 m*M)* was added to the perfusate (calculated to leave 1 n*M* free $Ca^{2+}$) it replaced an equimolar concentration of NaCl.

To inject chemicals into the cells, we filled the recording pipette with the following solutions: $Ca(OH)_2$ (90 m*M*); EGTA (100 m*M*) and Tris (100 m*M*) pH 4.6 or alternatively the $Ca(OH)_2$ was replaced by KOH (180 m*M*) in the recording pipette (Lisman and Brown 1972). The low pH (of 4.6) was required in order to dissolve the $Ca(OH)_2$.

*Recordings and stimulation.* Conventional methods for intracellular recording and for stimulation of barnacle photoreceptors were used as described previously (Brown et al. 1970; Hillman et al. 1972). The recording pipette was a glass microelectrode of 40 MΩ resistance when filled with 2 *M* KCl or 120–200 MΩ when filled with the injecting solutions. Injection (via a bridge circuit) of $Ca^{2+}$ or $K^+$ was performed by passing 0.1–0.3 nA positive current. EGTA was injected by passing 0.1–0.3 nA negative current out of the K-EGTA electrode. The membrane potential was constantly recorded on an oscilloscope, and a pen recorder. The light responses were sampled at 2 kHz, displayed by an IBM-AT computer and graphics accelerator card (CODAS1, Dataq Instruments) and stored on the hard disc of the computer for later analysis. The light stimulus came from a 12 V 100 W halogen lamp in conjunction with Ditric neutral density filters (Ditric Optics, Inc., Marlboro, MA, USA) and 3 KG3 (Schott) heat filters. The ocelli were stimulated via a 4 mm diameter light guide.

The unattenuated white light intensity, which was used in all experiments, at the level of the eye was 9.2 $mW/cm^2$.

## Results

### *Extracellular $La^{3+}$ reversibly abolishes the light response*

In order to make our measurements comparable to those obtained in flies (Hochstrate 1989) we used artificial sea water (ASW) with low (0.1 m*M)* $Ca^{2+}$ concentration to which $La^{3+}$ was added. Figure 1A shows the typical receptor potential of the barnacle photoreceptors in response to intense 10 s continuous light. Lowering $Ca^{2+}$ in the bathing medium to 0.1 m*M* led to an increase in the amplitude of the receptor potential at all but the lowest light intensities. The lower $Ca^{2+}$ level also slowed down the kinetics of the light responses and eliminated the initial transient phase of the response to intense light. Similar observations resulting from a reduction in $[Ca^{2+}]_i$ by several means have been reported before in the barnacle (Brown et al. 1970) and other invertebrate preparations (*Limulus,* Brown and Lisman 1975; fly, Muijser 1979; Minke 1982). These changes in the response waveform are at least in part due to a reduction in the effect of light adaptation which is initiated by an increase in cytosolic $Ca^{2+}$.

When $La^{3+}$ (1 m*M*) was applied to the bathing solution, a large reduction in the amplitude of the response to a constant intense test light was observed (Fig. 2A). The receptor potential was completely abolished 12 min after $La^{3+}$ application (Fig. 2A) even in response to a maximal intensity light pulse. The light response recovered fully 77 min after washing in $La^{3+}$ free ASW (Fig. 2B). The inset of Fig. 2A shows, using bridge measurements, that the elimination of the light response by $La^{3+}$ is accompanied by elimination of the light-induced conductance increase. Similar elimination of the light-induced conductance change was obtained in the presence of external EGTA (see below).

The effects of lower concentrations of $La^{3+}$ (0.1 or 0.5 m*M*) were also examined. Application of 0.1 m*M* $La^{3+}$ slightly increased the amplitude of the receptor potential. Application of 0.5 m*M* $La^{3+}$ *increased* significantly the response amplitude to a test light in all cells tested by 17.8±3.6% (Standard Error of the Mean, 4 cells). The enhancement of response amplitude by low concentration of $La^{3+}$ suggests that $La^{3+}$ affects two antagonistic processes (see Discussion).

The effect of $La^{3+}$ was sometimes accompanied by a slow depolarization of the membrane potential in the dark (up to 15 mV in some cells). The average resting potential of a random fraction of cells tested was −55±12.8 mV (SEM, 15 cells). The application of 1 m*M* $La^{3+}$ resulted, in all cells tested (14 cells in 14 barnacles), in a complete (but reversible) elimination of the light response even to the maximal intensity white light.

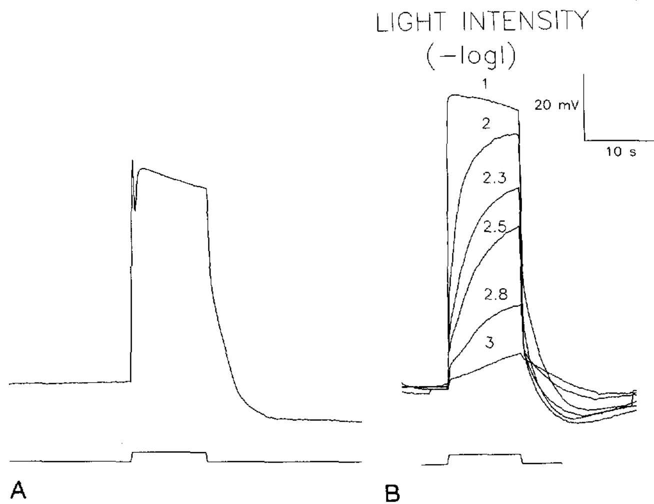


Fig. 1A, B. The waveform of the receptor potential recorded intracellularly from a single photoreceptor of a barnacle lateral ocellus. A The typical receptor potential in response to intense light was recorded ($-\log I = 1.0$) during perfusion with ASW containing 10 m$M$ $Ca^{2+}$. B A family of receptor potentials of a cell (perfused by ASW containing 0.1 m$M$ $Ca^{2+}$), in response to increasing intensity of white light stimuli with relative intensity as indicated

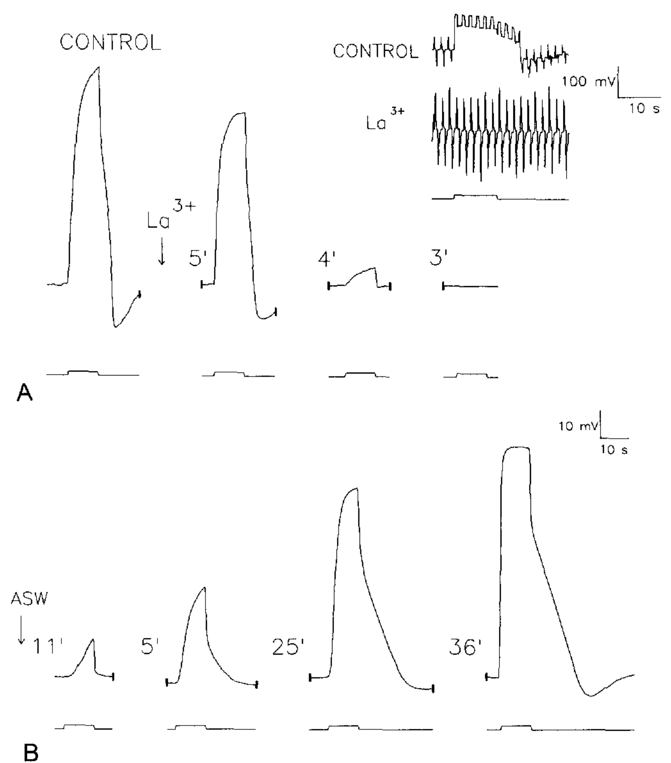


Fig. 2A, B. Lanthanum (1 m$M$ in 0.1 m$M$ $Ca^{2+}$-ASW) abolished reversibly the receptor potential. A The left response is a control response in ASW. The *arrow* indicates the time when $La^{3+}$ was applied. Five, 4 and 3 min pauses in the recordings between the light responses are indicated. *Inset:* Bridge measurements of conductance change before, during and after illumination ($-\log I = 1.0$). *CONTROL* (upper trace): A large increase in conductance was observed during and shortly after light. Application of 1 m$M$ $La^{3+}$ to the perfusate resulted in complete elimination of the conductance change 13 min after $La^{3+}$ application. B Recovery of the light response after 0.1 m$M$ $Ca^{2+}$-ASW was applied again to the same cell. The left trace was recorded 11 min after $La^{3+}$ removal and breaks in the recorded trace indicated 5, 25, and 36 min pauses. The light intensity of the constant test light was $-\log I = 2.0$

A relatively fast elimination of the light response by $La^{3+}$ required that several light pulses be given during $La^{3+}$ application. Application of $La^{3+}$ in the dark (20 min) without any illumination did not abolish the response to the test light but reduced its amplitude to about 10–15% of maximum and modified its waveform to a transient response (5 cells). Illumination was required to abolish the response in the presence of $La^{3+}$ completely within 20 min. Incubation of the cells in the dark in the presence of $La^{3+}$, for periods longer than 20 min, resulted in failure of the response amplitude to recover from the effect of $La^{3+}$ and in distortion of the response waveform.

*Extracellular EGTA abolished reversibly the light response*

The effect of $La^{3+}$ shown in Fig. 2 may have arisen from blocking of $Ca^{2+}$ influx into the barnacle photoreceptors. Another way to affect $Ca^{2+}$ influx into the cells is to reduce its concentration in the bathing solution by $Ca^{2+}$ chelating agents.

Figure 3 shows the effect of adding 7.5 m$M$ EGTA to the perfusate (0.1 m$M$ $Ca^{2+}$ ASW; resulting in less than 50 n$M$ of free $Ca^{2+}$). The light response was greatly reduced 2 min after EGTA application and 4 additional min in the presence of EGTA–ASW resulted in almost complete elimination of the response (Fig. 3). Two min later the response to the maximal light intensity was eliminated. The response recovered completely within 12 min after 0.1 m$M$ Ca ASW was perfused again (Fig. 3). Some variability in the time required to eliminate the response was observed in different barnacles. Nevertheless, the exposure to EGTA–ASW, like the ex-

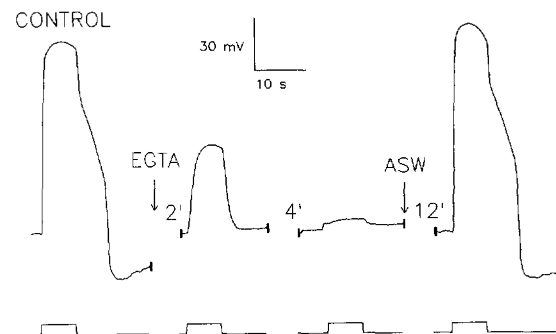


Fig. 3. EGTA (7.5 m$M$) in 0.1 m$M$ Ca-ASW reversibly eliminated the light response. *Left response:* control response to the constant test light ($-\log I = 2.0$). The two traces in the middle show responses to the test light 2 and 4 min after application of EGTA. The right trace shows the recovery of the response 12 min after 0.1 m$M$ $Ca^{2+}$-ASW was applied again

posure to $La^{3+}$, completely (and reversibly) eliminated the response to maximal intensity white light in all cells tested (12 cells in 12 barnacles).

The combination of EGTA and light abolished the light response within 15 min. Longer exposure to EGTA *without* illumination greatly reduced (to 10–20% of control) but did not abolish the light response even after perfusion for 20 min in the dark (data not shown). Longer (> 20 min) perfusion with EGTA usually resulted in failure of the response to recover.

### *Injection experiments*

The elimination of the light response by external $La^{3+}$ and EGTA suggests that extracellular $Ca^{2+}$ is required to induce the light response. To support the hypothesis that the reduced response resulting from application of $La^{3+}$ and EGTA is due to diminished influx of $Ca^{2+}$, we injected $Ca^{2+}$ iontophoretically during perfusion with $La^{3+}$ and EGTA.

To inject $Ca^{2+}$ or EGTA iontophoretically into the cells we used a recording pipette filled with the same solutions used to inject the above ions by Lisman and Brown (1972) and by Bolsover and Brown (1985) into *Limulus* ventral photoreceptors (see Materials and methods). Shortly (< 1 min) after penetration into the cell with the $Ca^{2+}$ and EGTA containing pipette, a positive (or negative) current of 0.1–0.3 nA was injected continuously into the cell via a bridge circuit for the remainder of the experiment. In most cases, stable voltage recordings were obtained throughout, data from the minority of experiments, in which the resistance of the electrode changed during the recording/injecting procedure, were discarded. The applied constant positive current reduced the amplitude of the maximal receptor potential by about 30% due to reduction in the driving force of the receptor potential. We cannot exclude the possibility that this depolarization of the photoreceptor's membrane activated voltage-sensitive $Ca^{2+}$-channels. Therefore, we performed control experiments in which $K^{+}$ was injected into the cells by similar current. The advantage of using a single electrode in such experiments is that minimal damage was caused to the recorded photoreceptors which gave maximal responses with amplitudes ranging between 70–100 mV before injection after dark adaptation.

Typically we recorded 3–4 light responses (every 2 min) to make sure that a constant receptor potential amplitude was obtained. The immediate effect of $Ca^{2+}$ injection was to speed up the kinetics of the receptor potential relative to the effect of $K^{+}$ injection (Fig. 4A, B, CONTROL). Application of 7.5 m*M* EGTA to the perfusate during $Ca^{2+}$ injection caused only a slight reduction in the peak receptor potential 9 min after application of EGTA (Fig. 4A) while a much larger reduction in steady-state amplitude was observed after 19 min (Fig. 4A). This result was verified in each of the 5 other cells tested. In contrast, injection of $K^{+}$ into the cell during perfusion with EGTA (7.5 m*M*) (in another barnacle – see Materials and methods) resulted in a fast and large reduction of the receptor potential in a similar manner to the results without iontophoretic injection (Fig. 3), only a very small transient response remained. This control experiment indicated that the depolarization induced by the positive current did not introduce appreciable amount of $Ca^{2+}$ into the cell via voltage-dependent $Ca^{2+}$ channels. The amplitude but not the waveform of the response recovered ~44 min after removal of EGTA from the perfusate. Recovery of the response waveform was observed 32 min later (data not shown). A similar result was obtained in each of the 6

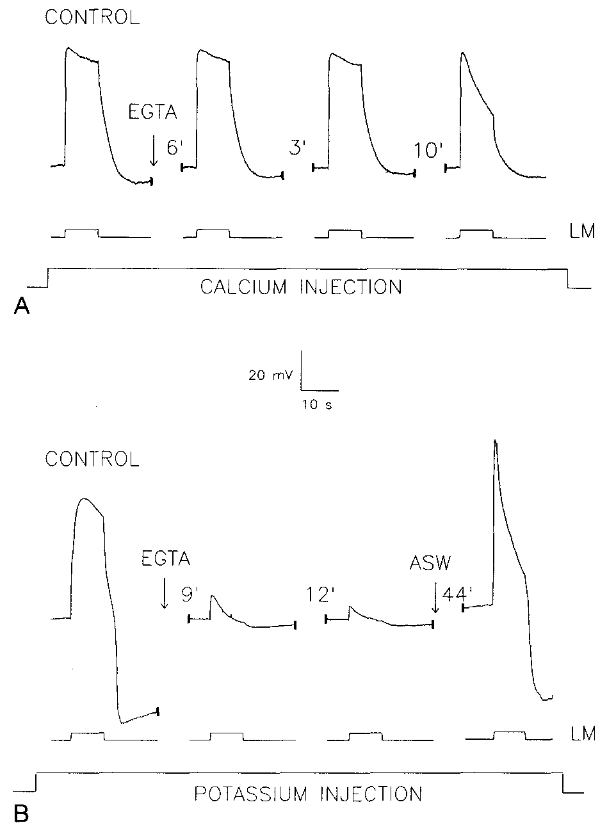


**Fig. 4A, B.** Intracellular iontophoretic injection of $Ca^{2+}$ but not $K^{+}$ protected the receptor potential from elimination by external EGTA. **A** The recording/injecting electrode contained 90 m*M* $Ca(OH)_2$, 100 m*M* EGTA and 100 m*M* Tris base buffer, pH 4.6. Positive current (0.3 nA) was applied immediately after penetration. The left response *(CONTROL)* was recorded during current injection (8 min after the beginning of injection). EGTA (7.5 m*M*) in 0.1 m*M* Ca-ASW was applied *(arrow)* and responses to the constant test light ($-\log I = 2.0$) were recorded 6, 9 and 19 min after EGTA application. **B** Intracellular recording in another barnacle. The recording/injecting pipette contained 180 m*M* KOH, 100 m*M* EGTA and 100 m*M* Tris base pH 4.6. Positive 0.3 nA current was injected immediately after penetration. The left response *(CONTROL)* was recorded 8 min after the beginning of the current injection. The large hyperpolarization after light offset is due to strong activity of the electrogenic pump (Koike et al. 1971). Nine min after application of EGTA *(arrow)* the light response to the test light ($-\log I = 1.0$) was largely reduced. It was almost eliminated 3 min later. The response amplitude, but not waveform recovered 44 min after 0.1 m*M* $Ca^{2+}$-ASW was applied again *(right trace)*. The bottom traces indicate the light monitor (*LM*) and $K^{+}$ injection

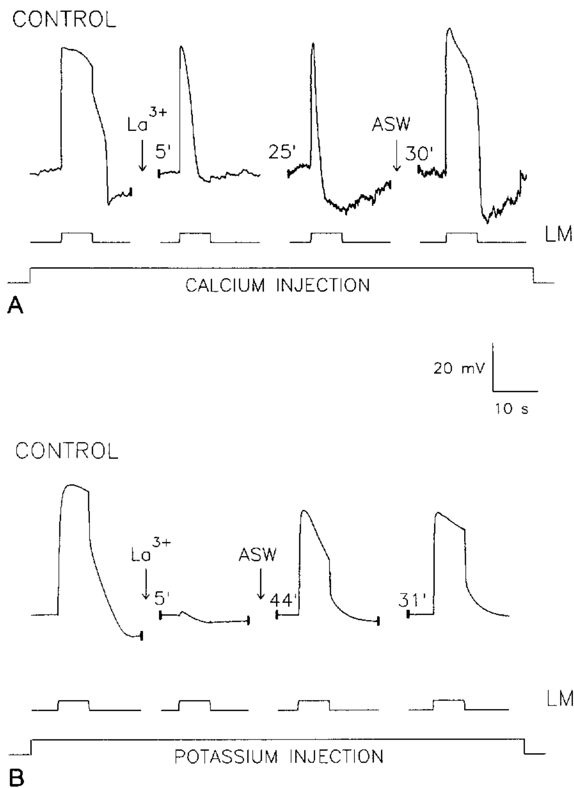


**Fig. 5.** Intracellular iontophoretic injection of $Ca^{2+}$ **(A)**, but not $K^+$ **(B)**, protected the initial amplitude (but not the steady state phase) of the light response from elimination by external $La^{3+}$. The experimental paradigm is similar to that of Fig. 4 except that $La^{3+}$ (1 m*M* in 0.1 m*M* $Ca^{2+}$-ASW) was applied instead of EGTA. The pauses between the recorded light responses are indicated in min. The constant test light was $-\log I = 1.0$

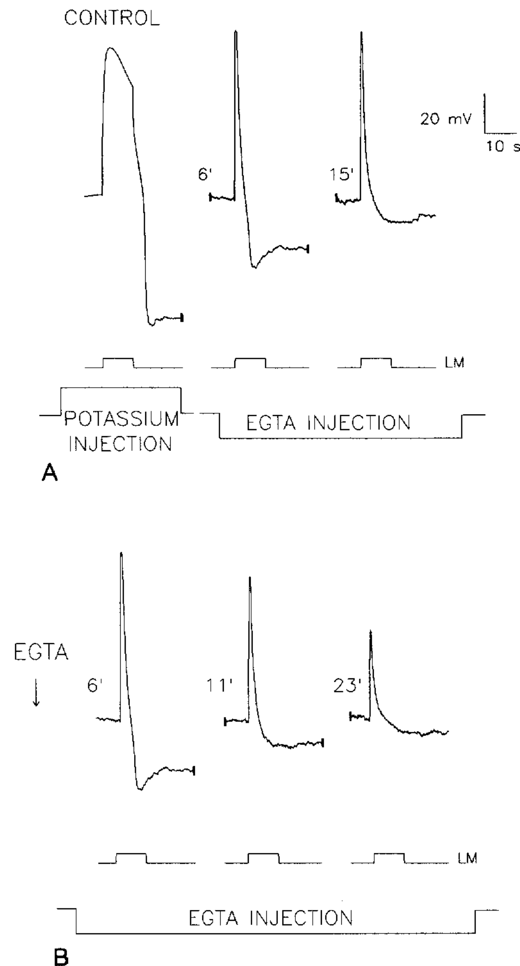


**Fig. 6A, B.** Intracellular iontophoretic injection of EGTA caused a decline in the light response during intense light. The recording/injecting pipette contained 180 m*M* KOH, 100 m*M* EGTA and 100 m*M* Tris base pH 4.6. **A** Positive current (0.2 nA) was injected during recording of the left trace *(CONTROL)*. The bottom trace indicates the time when the polarity of the injected current was reversed and a negative (0.3 nA) current was injected. Dark time of 60 s was required to obtain the transient responses after previous illumination. The intensity of the test light was $-\log I = 1.0$ for both **A** and **B**. The same barnacle of Fig. 4B was used for this experiment after recovery of the response waveform in ASW. **B** Addition of EGTA (7.5 m*M* in 0.1 m*M* $Ca^{2+}$-ASW) to the perfusate during EGTA injection did not abolish the receptor potential in a rapid manner similar to that observed without EGTA injection (see Figs. 3, 4B). After about 40 min the response amplitude was reduced to about 60% of the control level. The pause between the recorded light responses is indicated in min. The first response was recorded 6 min after application of 7.5 m*M* EGTA

barnacles used for the experiments. The large afterhyperpolarization during $K^+$ injection (Fig. 4B, CONTROL) is not uncommon in the barnacle photoreceptors, and is believed to be due to the activity of the electrogenic Na-K pump (Koike et al. 1971).

Figure 4 thus demonstrates that injection of $Ca^{2+}$, but not $K^+$, into the photoreceptors protected the transient phase of the receptor potential from elimination by external EGTA for a long time and protected the steady state phase for a limited time ($\sim$10 min).

Figure 5 shows experiments similar to Fig. 4, except that 1 m*M* $La^{3+}$ was applied to the perfusate instead of EGTA. Injection of $Ca^{2+}$ during $La^{3+}$ application protected the initial amplitude of the response but not the steady state phase, which declined to below baseline during illumination (Fig. 5A). The transient response recovered within one min in the dark during $La^{3+}$ application (data not shown). This transient response persisted throughout the $La^{3+}$ application and its transient waveform slowly changed back to normal after ASW was applied again. This result was observed in each of the additional 5 cells tested. Injection of $K^+$ instead of $Ca^{2+}$ during $La^{3+}$ application resulted in a fast and almost complete elimination of the response (both transient and steady state, Fig. 5B). The response amplitude and waveform largely recovered 75 min after $La^{3+}$ was removed from the perfusate (Fig. 5B). This result was repeated in 4 additional cells tested.

A transient light response, similar to that obtained during $Ca^{2+}$ injection in the presence of $[La^{3+}]_o$, could also be obtained in ASW when EGTA was injected into the cell by a negative current (K–EGTA electrode) (Fig. 6A, 5 cells tested). The transient response was obtained only during intense light and the rate of decline in-

creased with increasing light intensity like the *trp* phenotype.

Interestingly, application of EGTA (7.5 m*M*) to the perfusate during EGTA injection did not result in the rapid elimination of the transient light response but only in a slow reduction in amplitude; even after 40 min, the transient response amplitude was still about 60% of maximum (Fig. 6B). This is in sharp contrast to the effect of external EGTA without intracellular EGTA injection (Figs. 3 and 4B and see Discussion).

## Discussion

The results suggest that $Ca^{2+}$ is necessary for light excitation in the barnacle photoreceptors. The fact that steady injection of $Ca^{2+}$ was able to protect the transient phase and for a limited time also the steady-state phase against response elimination by external EGTA implies that, at least for the transient phases, it is a steady supply of $Ca^{2+}$ from any source, rather than specifically the rapid influx associated with the light response, that is needed for phototransduction. This fact furthermore suggests that some re-supply of $Ca^{2+}$, either to fill a still undescribed internal store or to maintain some minimal resting level is required for excitation. This suggestion is consistent with recent studies of H.M. Brown and colleagues (Brown et al. 1988; Brown and Rydqvist 1990) showing that an increase in $[Ca]_i$, either by direct pressure injection or by application of external DMSO depolarizes the barnacle photoreceptors by inducing inward current with a reversal potential similar to the light response.

Lanthanum is known to compete with $Ca^{2+}$ on binding sites of Ca-binding proteins (Nathan et al. 1988). It inhibits influx of $Ca^{2+}$ into cardiac cells (Wendt-Gallitelli and Isenberg 1985; Nathan et al. 1988) or presynaptic terminals (Miledi 1971). It also blocks Na-Ca exchange in vertebrate rods (Yau and Nakatani 1984). $La^{3+}$ also affects membrane surface charges (Frankenhaeuser and Hodgkin 1957) and, consequently, affects the surface membrane in a non-specific manner when applied in high concentration to the extracellular space.

The elimination of the receptor potential by external $La^{3+}$ in the bathing solution may arise from a direct block of all the light-sensitive channels. Alternatively, on the assumption that an increase in intracellular $Ca^{2+}$, mediated by a release from the $Ca^{2+}$ stores is necessary for light excitation, it might arise indirectly by blocking $Ca^{2+}$ entry into the photoreceptor which replenishes the intracellular $Ca^{2+}$ stores.

Blocking the light-sensitive channels by $La^{3+}$ cannot explain the total elimination of the light response because injection of $Ca^{2+}$ into the cells protected the initial phase of the light response in the presence of $[La^{3+}]_o$ and only the steady state phase was abolished during illumination. This result can be explained by analogy to the effect of $La^{3+}$ on the light-induced current (LIC) measured in *Drosophila* photoreceptors. In *Drosophila*, $La^{3+}$ blocks selectively one class of light-activated channels, with large $Ca^{2+}$ permeability, required for the steady state phase of the LIC leaving unaffected other channels with a reduced $Ca^{2+}$ permeability (Hardie and Minke 1992). Perhaps, the barnacle photoreceptors have also at least two classes of light-activated channels but only the channels permeable mainly to $Ca^{2+}$ are blocked by $La^{3+}$.

Strong support for the hypothesis that $Ca^{2+}$ is necessary for excitation came from the total elimination of the receptor potential by EGTA. This effect cannot be explained by some damage caused to the light-sensitive channels by EGTA because injection of $Ca^{2+}$ into the cells during external EGTA application protected the receptor potential as it does in *Limulus* ventral photoreceptors (Bolsover and Brown 1985). This result is consistent with the hypothesis that $La^{3+}$ abolishes the light response via blocking $Ca^{2+}$ influx. Furthermore, it suggests that $Ca^{2+}$ influx has a dual role in excitation: i) To produce directly at least part of the receptor potential. ii) To replenish internal $Ca^{2+}$ in some hypothetical $Ca^{2+}$ stores which are required to produce the transient phase of the light response, a phase that can be protected from elimination by $La^{3+}$ during calcium injection. The results did not give a clear answer as to the role of $Ca^{2+}$ entry during prolonged light. The steady state phase need not be mediated by $Ca^{2+}$ current if $Ca^{2+}$ injection during EGTA protects this phase. However, this protection is rather limited in time ($\sim$10 min). It is interesting to note that a total elimination of the light response by external EGTA and $La^{3+}$ was obtained only after illumination, suggesting that the complete block of phototransduction is obtained via reducing $[Ca^{2+}]_i$ in light-sensitive intracellular compartments with a very limited capacity and which needed to be replenished from the extracellular space.

The similarity in the effect of $La^{3+}$ and the *trp* or *nss* mutations in the fly suggest that $La^{3+}$ and the above mutations block a $Ca^{2+}$ transporting protein located at the surface membrane. This $Ca^{2+}$ channel transporter may be activated by a reduction of $[Ca^{2+}]$ in the Ins(1, 4, 5)$P_3$-sensitive $Ca^{2+}$ pools during illumination (Suss-Toby et al. 1991). Furthermore, we suggested that the difference in the action of $La^{3+}$ in the fly and *Limulus* may arise from a different mechanism of $Ca^{2+}$ mobilization in the two species. The barnacle, in which most of the intracellular change in $Ca^{2+}$ results from an influx from the extracellular space (Brown and Blinks 1974; Brown et al. 1988) provides an important preparation for testing the above hypothesis. The present study supports the hypothesis that the differences in the action of $La^{3+}$ on the receptor potential in *Limulus*, fly and barnacle can be explained by assuming different sizes of the internal $Ca^{2+}$ stores: Large stores in *Limulus* with efficient mobilization of $Ca^{2+}$ between the various $Ca^{2+}$ pools and medium and small stores in the fly and barnacle, respectively.

One of the striking (but difficult to explain) observations in our experiments was the modification of the receptor potential into a transient response when EGTA was injected into the cell and the relative inefficiency of

external EGTA in abolishing the transient response under these conditions. This transient response is similar to that observed in the *trp* or *nss* mutants of the fly and in wild type fly in the presence of external $La^{3+}$. We still do not have a satisfactory explanation for this observation. Another surprising phenomenon is the increase in response amplitude during low external $La^{3+}$. This observation may be explained by a partial block of light-activated $Ca^{2+}$ influx leading to a smaller than usual increase in intracellular $Ca^{2+}$, causing a reduction in light adaptation but not in excitation. These two antagonistic processes are both mediated by an increase in internal $Ca^{2+}$ but presumably with a different dependence on $Ca^{2+}$ concentration (Walz 1992). Support for this suggestion has been provided by H.M. Brown and colleagues (Brown et al. 1988; Brown and Rydqvist 1990) who show that pressure injection of $Ca^{2+}$ into the barnacle photoreceptor which increased $[Ca]_i$ by 0.5 $\mu M$ or more desensitizes (adapts) the light response while an increase of $[Ca]_i$ to a smaller level of about 0.3 $\mu M$, using DMSO, facilitates (increases) the light response. The existence of two antagonistic processes, both mediated by $Ca^{2+}$, may account for the relatively high concentration of $La^{3+}$ required for inhibition of the light response.

## Conclusion

We have demonstrated, by a reversible elimination of the receptor potential of the barnacle photoreceptors with extracellular EGTA and, by protecting the response amplitude, with $Ca^{2+}$ injection, that a rise in intracellular $Ca^{2+}$ is necessary for light excitation in the barnacle photoreceptor. We have furthermore demonstrated that external $La^{3+}$ reversibly blocks the light response while injection of $Ca^{2+}$ protected the transient, but not the steady state phase of the response suggesting that $La^{3+}$-sensitive inward current is necessary to maintain the steady state phase of the receptor potential during intense light. This current presumably replenishes the internal $Ca^{2+}$ stores needed to induce the transient and perhaps part of the steady state phase of the light response.

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