The Light-sensitive Conductance of Hyperpolarizing Invertebrate Photoreceptors: A Patch-Clamp Study

MARIA DEL PILAR GOMEZ and ENRICO NASI

From the Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Tight-seal recording was employed to investigate membrane currents in hyperpolarizing ciliary photoreceptors enzymatically isolated from the eyes of the file clam (*Lima scabra*) and the bay scallop (*Pecten irradians*). These two organisms are unusual in that their double retinas also possess a layer of depolarizing rhabdomeric cells. Ciliary photoreceptors from *Lima* have a rounded soma, 15–20 μm diam, and display a prominent bundle of fine processes up to 30 μm long. The cell body of scallop cells is similar in size, but the ciliary appendages are modified, forming small spherical structures that protrude from the cell. In both species light stimulation at a voltage near the resting potential gives rise to a graded outward current several hundred pA in amplitude, accompanied by an increase in membrane conductance. The reversal potential of the photocurrent is ≈ −80 mV, and shifts in the positive direction by ~39 mV when the concentration of extracellular K is increased from 10 to 50 mM, consistent with the notion that light activates K-selective channels. The light-activated conductance increases with depolarization in the physiological range of membrane voltages (−30 to −70 mV). Such outward rectification is greatly reduced after removal of divalent cations from the superfusate. In *Pecten*, cell-attached recordings were also obtained; in some patches outwardly directed single-channel currents could be activated by light but not by voltage. The unitary conductance of these channels was ≈26 pS. Solitary ciliary cells also gave evidence of the post stimulus rebound, which is presumably responsible for initiating the "off" discharge of action potentials at the termination of a light stimulus: in patches containing only voltage-dependent channels, light stimulation suppressed depolarization-induced activity, and was followed by a strong burst of openings, directly related to the intensity of the preceding photostimulation.

INTRODUCTION

Physiological investigations of visual excitation in invertebrates have focused mostly on depolarizing rhabdomeric cells, which are the predominant type of photoreceptors in these organisms. The eyes of several invertebrate species, however, appear to possess cells that utilize fundamentally different effector mechanisms (for a review,

Address correspondence to M. Gomez or E. Nasi, Department of Physiology, Boston University School of Medicine, 80 East Concord Street, Boston, MA, 02118.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/94/06/0939/18 $2.00
Volume 103 June 1994 939–956

see McReynolds, 1976). The best known example is found in the retina of the scallop, *Pecten irradians.* Early anatomical observations by Dakin (1910) had revealed the presence of two distinct layers of cells—both presumed to be sensory—projecting their axons centripetally through separate branches of the optic nerve. Subsequently, Hartline (1938) demonstrated that while the proximal branch of the nerve produced light-induced neuronal discharges, in fibers originating from the distal retinal layer light stimulation caused suppression of action potentials; this phenomenon was thought to reflect "sensory inhibition" induced by the photostimulation. In support of such notion, intracellular recordings in the intact retina of *Pecten* revealed that in some cells light evoked membrane depolarization, while in others a *hyperpolarization* was observed (Gorman and McReynolds, 1969; McReynolds and Gorman, 1970*a*). In cells of the latter category the resting potential ranged from −20 to −40 mV, and the hyperpolarizing photoresponses were shown to be mediated by a light-induced increase in membrane conductance to potassium ions (McReynolds and Gorman, 1970*b*).

"Off" neuronal discharges to light were also reported in the optic nerve of several other bivalve mollusks, such as *Cardium edule* (Barber and Land, 1967) and *Lima scabra* (Mpitsos, 1973), and also in nerve fibers of mollusks that lack a differentiated eye and specialized photoreceptor cells, such as *Spisula solidissima* (Kennedy, 1960) and *Mercenaria mercenaria* (Wiederhold, MacNichol, and Bell, 1973). Intracellular recordings of light-induced membrane hyperpolarizations have been obtained from the giant clam *Tridacna* (Wilkens, 1984) and *Lima* (Mpitsos, 1973; Cornwall and Gorman, 1983); the eye of the latter organism shares a remarkable similarity with that of the scallop, in that it also contains a proximal layer of rhabdomeric depolarizing cells (Mpitsos, 1973; Nasi, 1991*a*). The morphological differences between proximal and distal cells are striking, both in *Lima* and in *Pecten;* in the scallop, electron micrographs reveal microvilli-covered lobe in proximal cells, whereas distal cells display whorls of modified ciliary processes (Miller, 1958; Barber, Evans, and Land, 1967). Similarly, in fixed thick sections of *Lima* eyes, Bell and Mpitsos (1968) briefly described rhabdomeric photoreceptors and support cells in the eyecup proper, whereas in a distal structure (which had initially been mistaken for a lens) distinct bundles of cilia could be observed.

Positive identification of the cells giving rise to the hyperpolarizing responses by means of dye injection and subsequent histological examination was not performed in the studies cited above, partly because impalements of such small cells are technically difficult and usually short-lived. A number of considerations strongly argues, albeit indirectly, in favor of the notion that these responses are true receptor potentials: (*a*) in *Pecten* the spatial location of recording site (distal vs proximal) appeared to correlate with the polarity of the response observed (McReynolds and Gorman, 1970*a*). (*b*) No anatomical evidence of interconnections between distal and proximal cells has been found (Barber, Evans, and Land, 1967). (*c*) Light sensitivity in hyperpolarizing cells was reported to be lower than in depolarizing photoreceptors (McReynolds and Gorman, 1970*a*). (*d*) Hyperpolarizing photoresponses survived treatments designed to interfere with depolarizing receptor potentials and with the transmission of impulses to second-order neurons (e.g., perfusion with sodium-free solution and with tetrodotoxin; Toyoda and Shapley, 1967). Such arguments,

although forceful, may not yet constitute conclusive proof (see Ratliff, 1974, p. 227). A direct approach to eliminate potential confounding factors that may be present in the intact retina entails examining the electrophysiological behavior of isolated cells.

In previous reports, the eye of *Lima* was enzymatically and mechanically dissociated, and patch-electrode measurements were performed in a class of solitary cells bearing a distinct microvilli-covered lobe. Light was shown to evoke a complex-shaped depolarizing receptor potential (Nasi, 1991*a*), and voltage-clamp recording was employed to characterize both the voltage-dependent conductances (Nasi, 1991*b*) and the light-activated conductances (Nasi, 1991*c*). Patch-clamp recordings were also obtained from dissociated rhabdomeric photoreceptors from *Pecten*, and the properties of light-dependent single channels were characterized (Nasi and Gomez, 1992). In those studies, however, other types of visual cells could not be identified after dispersion of the retina. Modifications of the dissociation protocols recently proved successful in isolating ciliary photoreceptors from both *Lima* and *Pecten*. In this report, we examine macroscopic photocurrents in solitary ciliary cells from both organisms, and light-dependent single-channel currents from cell-attached patches in *Pecten*. Some of these results have been presented in a preliminary report (Gomez and Nasi, 1991).

METHODS

Isolation of Hyperpolarizing Photoreceptors

Specimens of *Lima scabra* were obtained through Carolina Biological Supply Co. (Burlington, NC) and maintained in an aquarium filled with artificial sea water (Instant Ocean, Aquarium Systems, Mentor, Ohio) at a temperature of 24°C. *Pecten irradians* were obtained from the Marine Resources facility at the Marine Biological Laboratory (Woods Hole, MA), and used immediately. The general protocol for dispersing the retina of these two mollusks has been described previously (Nasi, 1991*a*; Nasi and Gomez, 1992); slight modifications were introduced to increase the yield of ciliary cells, which appear more difficult than rhabdomeric cells to separate from the surrounding matrix of tissue. Briefly, 5–8 *Lima* eyecups dissected under dim red light illumination ($\lambda > 650$ nm) were incubated in collagenase (Worthington Type II, 2,000 U/ml [Worthington Biochemical Corp., Freehold, NJ]) for 50 min at 24°C, and subsequently in trypsin (Sigma Type III, 50,000 U/ml; Sigma Chemical Co., St. Louis, MO) for an additional 40 min. Isolated retinas of *Pecten* were treated with Pronase (Boehringer, 1,000 U/ml) for 50–60 min at 22°C. After washing in sea water supplemented with 3% fetal calf serum, the tissue was gently triturated with a fire-polished Pasteur pipette, and the resulting suspension transferred to the recording chamber. The flow-chamber was pre-treated with concanavalin A to promote cell adhesion, as previously described (Nasi, 1991*a*). The effectiveness of this procedure with ciliary cells proved more limited than for rhabdomeric photoreceptors, especially in the case of *Lima*. Other attachment substrates were also tried, including poly-D-lysine, laminin, and Cell-Tak® (Collaborative Research Inc., Bedford, MA), with no substantial improvement.

Artificial sea water (ASW) contained 480 mM NaCl, 10 mM KCl, 49 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM HEPES, and 5 mM glucose, pH 7.8 (NaOH). In high-potassium

ASW KCl was increased to 50 mM, replacing NaCl. In 0-divalent ASW $CaCl_2$ and $MgCl_2$ were omitted, and NaCl was increased to 570 mM. All experiments were conducted at room temperature (22–24°C).

Electrophysiological Recording

Patch electrodes were fabricated with borosilicate capillary tubing (type 7052, Garner Glass, Claremont, CA) and fire-polished immediately before use. For whole-cell recording thin-wall glass electrodes were employed (1.5 mm o.d., 1.1 mm i.d.), pulled to a tip diameter of 2–3 μm. The electrodes were filled with intracellular solution containing 100 mM KCl, 200 mM K-glutamate or K-aspartate, 12 mM NaCl, 5 mM Na_2ATP, 5 mM $MgCl_2$, 15 mM HEPES, 1 mM EGTA and 300 mM sucrose, pH 7.3; in some experiments 100 μM GTP was also included. The electrode resistance measured in sea water was 2–6 MΩ. In all recordings series resistance errors were corrected via a positive feedback circuit in the amplifier. For single-channel recording, thick wall electrodes (1.5 mm o.d., 0.75 mm i.d.) were filled with artificial sea water and had a resistance ranging from 6 to 12 MΩ. After seal formation, the resistive feedback in the head-stage of the patch-clamp amplifier was electronically switched from 100 MΩ to 50 GΩ. Currents were low-pass filtered with a Bessel 4-pole filter, using a cutoff frequency of 500–1,000 Hz for macroscopic current recordings, and 2,000 Hz for single-channel measurements. Records were digitized on-line at 2–10 KHz sampling rate (12-bit resolution) by an analog/digital interface board (model 2821, Data Translation, Marlboro, MA) installed in an IBM AT computer, and stored on Bernoulli cartridges (Iomega, South Roy, UT). Voltage- and light-stimuli were applied by a microprocessor-controlled programmable stimulator (Stim 6, Ionoptix, Milton, MA).

Two different arrangements were used for optical stimulation. In the first one, the light beam of an optical stimulator was combined with that of the microscope illuminator via a beam splitter prism placed above the condenser, as previously described (Nasi, 1991*a*,*c*; Nasi and Gomez, 1992). The second stimulator, designed to deliver a higher photon flux, consisted of a 75 W Xenon arc lamp (PTI, So. Brunswick, NJ), a 45° cold mirror ($\lambda < 690$ nm, Omega Optical, Brattleboro, VT) to eliminate IR, an electromechanical shutter (Vincent Associates, Rochester, NY) and a light guide (Schott Fiber Optics, Southbridge, MA) that was coupled to the epifluorescence port of an inverted microscope (Zeiss ICM 405). The light beam was reflected by a dichroic mirror ($\lambda < 650$ nm; Omega Optical), and focused onto the preparation through the microscope objective (Nikon 40× LWD, 0.65 n.a.). To provide a quantitative reference for light intensity in the two arrangements, the photocurrents elicited by flashes of monochromatic vs white light of different intensities from either optical stimulator were measured under voltage clamp in several cells (*Pecten:* $n = 3$; *Lima:* $n = 2$). Monochromatic light was obtained by interposing a three-cavity interference filter (540 nm peak, 10 nm half-width; Ditric Optics, Hudson, MA) in the Xenon beam path. Changes of stimulating light intensity in a given experiment are expressed as $\log_{10}(I/I_o)$, where I_o is the intensity of the unattenuated light beam. Calibrated neutral-density filters (Melles Griot, Irvine, CA) provided controlled attenuation. During experimental manipulations the cells were viewed with a Newvicon TV camera (model WV-1550, Panasonic, Secaucus, NJ) using a near-IR long-pass filter for illumination ($\lambda > 780$ nm; Andover Corporation, Salem, NH). The infrared illuminator was tuned off for several minutes before testing light responses.

RESULTS

In any retinal preparation of *Lima*, isolated ciliary cells can be distinguished from rhabdomeric cells by their morphology. A typical example is shown in the Nomarski

micrograph in Fig. 1 *A*. The cell body is nearly spherical and has a tuff of ciliary processes emanating from one side, that reach a length of 20–30 μm. These cells lack the dark screening pigment that is quite conspicuous in the rhabdomeric photoreceptors (Nasi, 1991*a*). Stumps of axons were never observed. A cell from *Pecten* is shown in Fig. 1 *B*. The cell body is similar in size to that of *Lima* ciliary photoreceptors, but displays rounded appendages, instead of the fine, long processes. These appendages are presumably formed by the whorls of membrane from modified cilia, first described by Miller (1958).

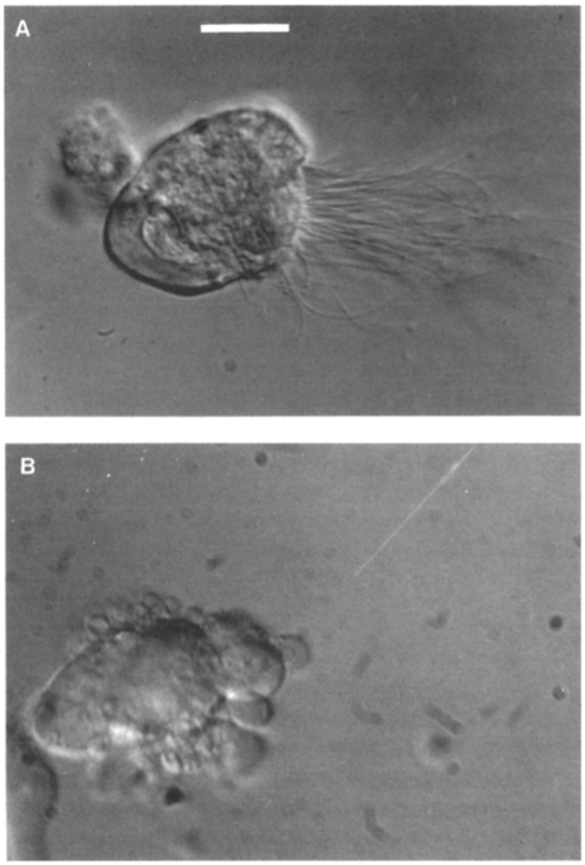

FIGURE 1. (A) Light micrograph of a ciliary photoreceptor dissociated from the eye of *Lima*, viewed under Nomarski differential-interference contrast optics (×100, oil-immersion). (*B*) Solitary ciliary cell from *Pecten* (Nomarski DIC, ×40 water-immersion). Bar, 10 μm.

Photoresponsiveness of isolated ciliary cells was tested under voltage clamp; light-induced changes in membrane conductance were monitored by superimposing a 50-Hz square-wave command onto the steady holding potential. Fig. 2 *A* shows the outward photocurrent in a *Lima* cell, elicited by a step of light delivered at −20 mV. The response is several hundred pA in amplitude. The large increase in the size of the current steps in response to the square-wave perturbation (10 mV peak-to-peak) shows that the photoresponse was accompanied by a marked increase in membrane conductance, from 1.2 to 29 nS. A similar recording from a *Pecten* cell is shown in Fig.

B: a nearly saturating photoresponse was elicited by a bright, 20 ms flash at −20 mV; the current jumps produced by the voltage steps (4 mV peak-to-peak) show that the membrane conductance increased from 12 to 63 nS at the peak of the photoresponse.

The effect of varying the intensity of stimulating light is shown in Fig. 3. Fig. 3 *A* (*left*) shows superimposed traces from an intensity series recorded in a *Lima* cell. A period of 1 min was interposed between flashes, which was sufficient to fully recover sensitivity to the dark-adapted level (data not shown). The peak current amplitude is plotted as a function of log relative light intensity on the right hand side. Fig. 3 *B* illustrates the responses of a *Pecten* cell tested in a similar way. For comparison, the

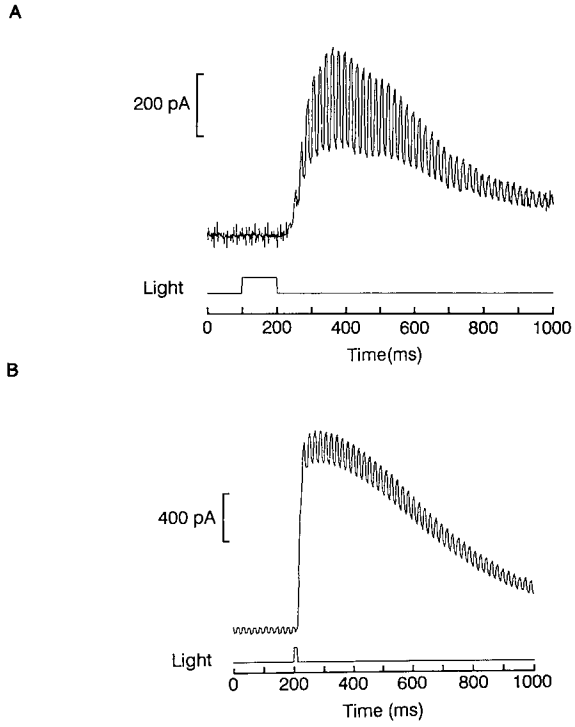

FIGURE 2. Outward photocurrent and membrane conductance changes in ciliary photoreceptors. (*A*) *Lima* cell voltage-clamped at −20 mV, with superimposed voltage steps (50 Hz, 10 mV p-p) to monitor the time course of input resistance changes. Light stimulus 100 ms, $1.5 \cdot 10^{14}$ photons·cm^{-2}·s^{-1}. (*B*) *Pecten* ciliary cell held at −20 mV stimulated with a 20 ms flash ($5 \cdot 10^{17}$ photons·cm^{-2}·s^{-1}), with voltage perturbation superimposed on the steady holding voltage (4 mV p-p, 50 Hz). In both *A* and *B* the photoresponse was accompanied by a marked increase in membrane conductance.

light intensity that evoked half-saturating inward photocurrents in dark-adapted rhabdomeric photoreceptors from the same retinal preparations was lower by ~2.4 log units for *Lima* (n = 3 and 5 for ciliary and rhabdomeric cells, respectively), and by 2.1 log units for *Pecten* (n = 3 for each type).

The reversal voltage of the light-induced current was determined by clamping the membrane to different steady voltages for several seconds before stimulating with light. Fig. 4 *A* shows recordings obtained from a *Lima* photoreceptor. When the membrane potential was hyperpolarized to −100 mV a barely detectable inward photocurrent was elicited. A similar experiment conducted in *Pecten* is shown in Fig. 4 *B;* the lowest trace reveals a small inward current at −100 mV. In Fig. 4 *C* the peak

amplitude of the response, normalized with respect to that measured at −40 mV, is plotted as a function of the holding potential for four *Lima* cells; the average value of the reversal voltages (determined by interpolation) was −78.5 mV ± 9.2 SD. In Fig. 4 *D* a similar plot is shown for *Pecten* cells; the average reversal voltage was −81.2 mV ± 2.9 SD (n = 5). Under the recording conditions employed (10 mM extracellular K, 300 mM K in the electrode) the calculated Nernst potential for potassium is −86 mV

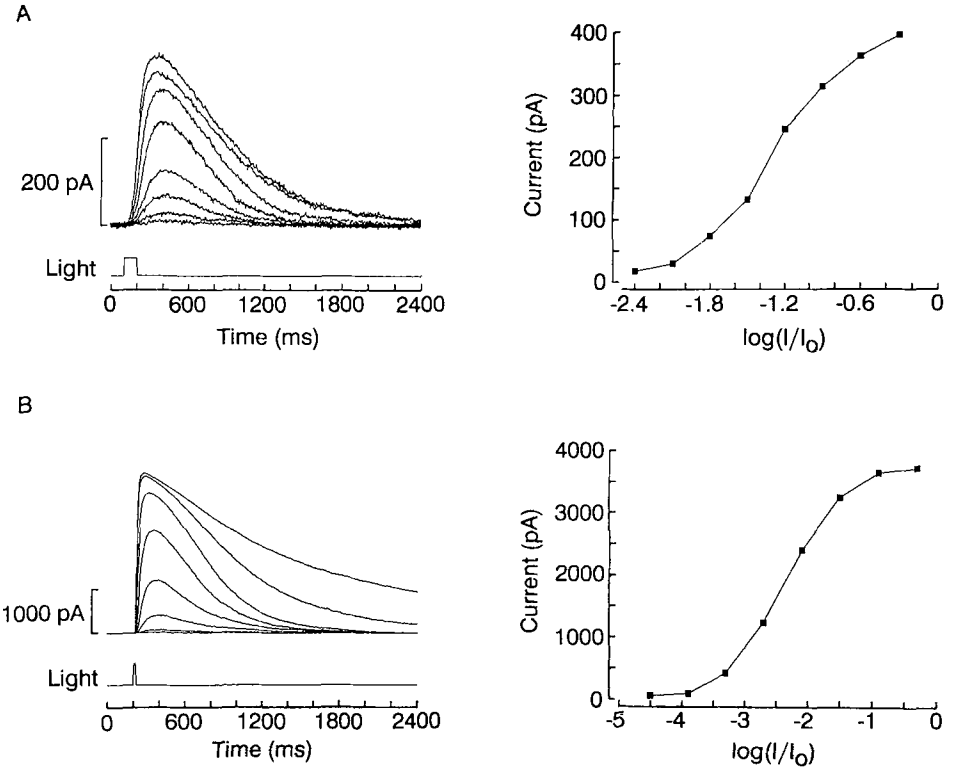

FIGURE 3. Effect of light-intensity on the photocurrent. (*A*) Photocurrent in a ciliary *Lima* photoreceptor voltage clamped at −20 mV. The intensity of the light steps, 100 ms in duration, was increased at 0.3 log increments. On the right, the peak amplitude of the photocurrent is plotted as a function of light attenuation. Unattenuated light intensity $2.4 \cdot 10^{15}$ photons·cm^{-2}·s^{-1}. (*B*) Intensity series in a *Pecten* ciliary cell with flashes 20 ms in duration, at −20 mV holding voltage. (*Right*) Plot of peak current vs log stimulus attenuation (Re: $5 \cdot 10^{17}$ photons·cm^{-2}·s^{-1}).

(assuming that the cytosolic compartment equilibrates with the electrode solution), in reasonable agreement with the observed values. In both species it proved difficult to obtain sizable light-induced inward currents at very negative voltages under normal ionic conditions: in several other cells tested (n = 5) the photocurrent nearly vanished at holding potentials below −70 mV, but did not become inward at more negative voltages (up to −90 mV). Larger hyperpolarizations in most cases were

deleterious (see for example increase in noise in the two bottom traces of Fig. 4 *A* and *B*) and lead to an irreversible increase in membrane leakage.

Fig. 5 shows that increasing the extracellular concentration of potassium to 50 mM shifted the reversal potential in the positive direction (ΔV_{rev} = 39.3 mV ± 2.0 SD, n = 4), in a way quantitatively comparable to the displacement of the Nernst potential for potassium ions (40.5 mV). In high-potassium solution inward photocur-

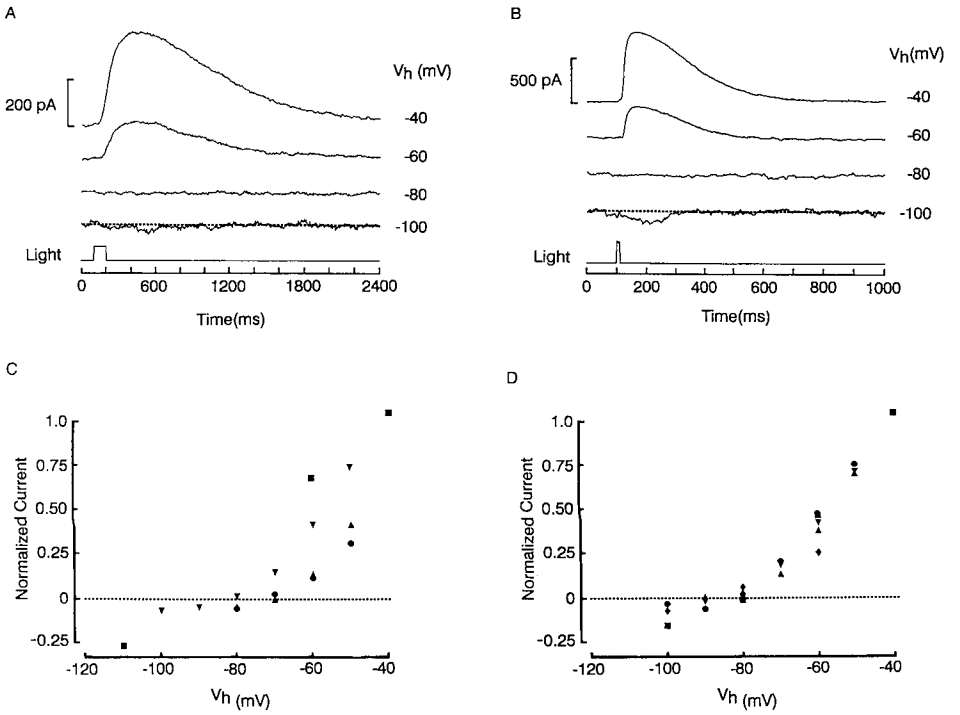

FIGURE 4. Reversal of the light-dependent current in ciliary photoreceptors. (*A*) *Lima*. Light stimuli 100 ms in duration, $1.5 \cdot 10^{14}$ photons·cm^{-2}·s^{-1}, delivered at 2-min intervals. The holding potential was stepped to the indicated levels ~5 s before each stimulus, then returned to −40 mV between trials. (*B*) *Pecten*. Similar procedure, except that shorter flashes were used (20 ms, $5 \cdot 10^{17}$ photons·cm^{-2}·s^{-1}). Large hyperpolarizations (e.g., *bottom current traces*) typically resulted in noisy records. (*C*) Relative amplitude of the light-induced current as a function of holding potential for four *Lima* photoreceptors (different symbols). (*D*) Normalized *I-V* plot for five *Pecten* cells. In *C* and *D*, values were normalized with respect to the peak photocurrent measured at −40 mV.

rents are easily measured close to the reversal voltage, but became smaller as the membrane was hyperpolarized further (n = 4). Stimuli subsequently delivered at less negative voltages elicited normal photocurrents, showing that the cell had not lost responsiveness. In Fig. 5 *D* the values of the light-induced membrane conductance change measured in 10 and in 50 mM external K are plotted as a function of the holding potential; as the membrane is hyperpolarized, the conductance decreases

monotonically in a similar way in the two ionic conditions. Such observation may account for the difficulty in detecting a reversal of the photoresponse.

The light-sensitive current of amphibian rods also displays a prominent outward rectification, which has been shown to be due to a voltage-dependent blockage of the channels by divalent cations (for a review, see Yau and Baylor, 1989). We investigated whether a similar mechanism is present in *Pecten* ciliary cells. Fig. 6 *A* shows a family

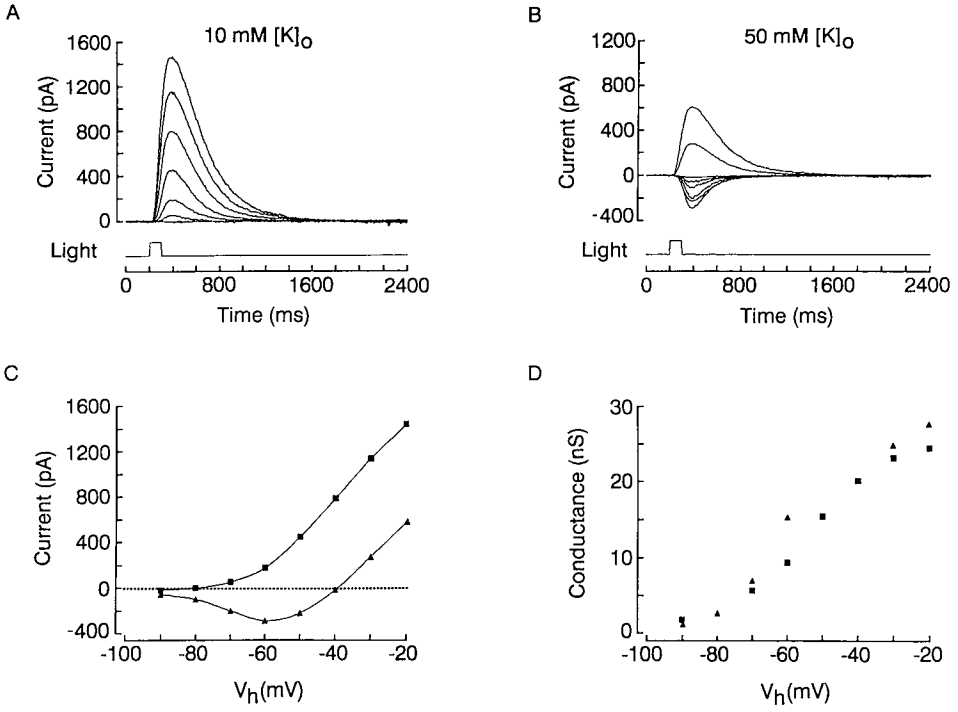

FIGURE 5. Dependency of the reversal potential on the concentration of extracellular potassium. A *Pecten* cell was voltage clamped at steady potentials in the range −20 to −90 mV in normal ASW containing 10 mM potassium (*A*). The photocurrent reversed at −80.1 mV (see *C*, *squares*). After switching the perfusate to a solution containing 50 mM K (*B*) the photocurrent reversal shifted by 39.2 mV in the positive direction (*C*, *triangles*). As the membrane was hyperpolarized beyond −60 mV, the amplitude of the inward light-evoked current decreased. (*D*) Light-induced conductance (calculated according to the formula $g = I/(V_m - V_{rev})$ as a function of membrane voltage in ASW (*squares*) and high-K solution (*triangles*). Light intensity $4.2 \cdot 10^{14}$ photons·cm^{-2}·s^{-1}.

of photocurrent traces elicited by a light stimulus of fixed intensity at different voltages (−20 to −90 mV) in ASW and after switching to a solution lacking both calcium and magnesium. The size of the photocurrent is increased in 0-divalent solution; moreover, the response below the reversal potential is greatly enhanced. Fig. 6 *B* shows that the outward rectification observed in ASW is virtually eliminated in 0-divalent solution, and the current-to-voltage relation becomes linear.

On-cell patch recording from *Lima* ciliary cells failed to yield single-channel currents activated by light. One plausible explanation is that the light-sensitive conductance may be confined to the ciliary processes, where the photopigment molecules are presumed to be located. In *Limulus* ventral photoreceptors, for example, both rhodopsin and light-activated channels are spatially segregated in the

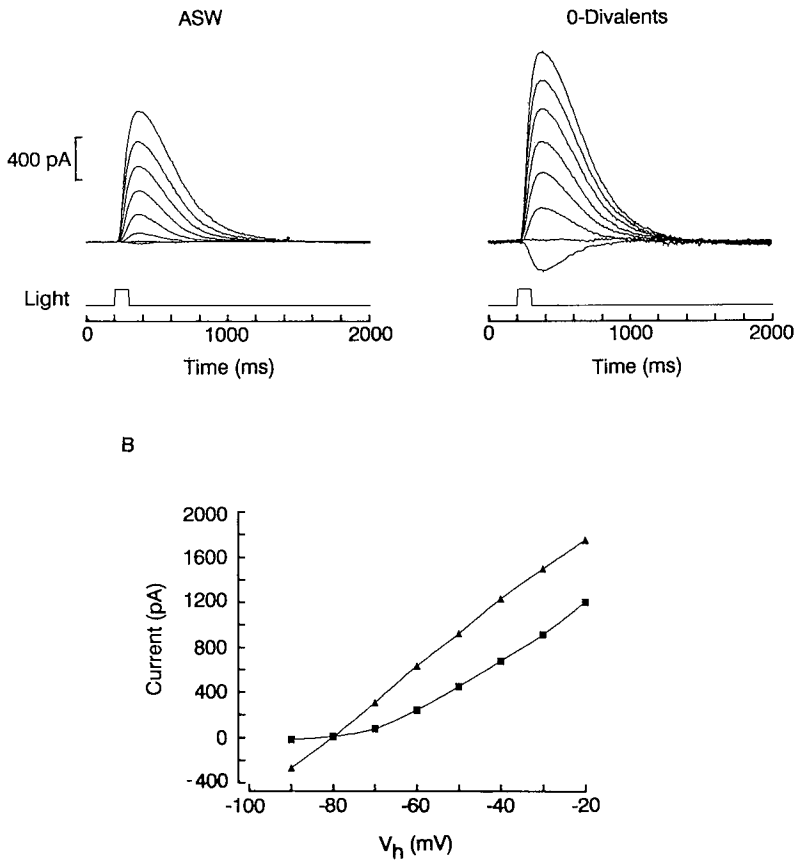

FIGURE 6. Effect of removal of divalent cations on the rectification of the photocurrent. A *Pecten* ciliary photoreceptor was stimulated with flashes of light (100 ms, $4.2 \cdot 10^{14}$ photons·cm^{-2}·s^{-1}) while the membrane potential was clamped between −20 and −90 mV. The recording chamber was perfused with either ASW containing 10 mM Ca and 49 mM Mg (*A, left*), or a solution in which divalent cations had been omitted (*A, right*). Removal of Ca and Mg increased the size of the light-evoked currents and eliminated the outward rectification. In *B*, the amplitude of the photocurrent in ASW (*squares*) and in 0-divalents (*triangles*) is plotted as a function of membrane potential.

microvilli-covered rhabdomeric membrane (Fein and Charlton, 1975; Stern, Chinn, Bacigalupo, and Lisman, 1982). Unfortunately, tight-seal recordings were not obtained from the fine cilia of *Lima* photoreceptors, owing to their forbidding geometry. *Pecten* cells appeared more favorable in this respect because the individual ciliary appendages are flattened above the stalk, forming lamellae that wrap around

one another (Miller, 1958; Barber et al., 1967) giving rise to more bulbous structures (see Fig. 1 *B*).

On several occasions, on-cell patch recordings in ciliary-type *Pecten* cells showed channel currents activated by light stimulation (n = 13). Fig. 7 *A* shows traces

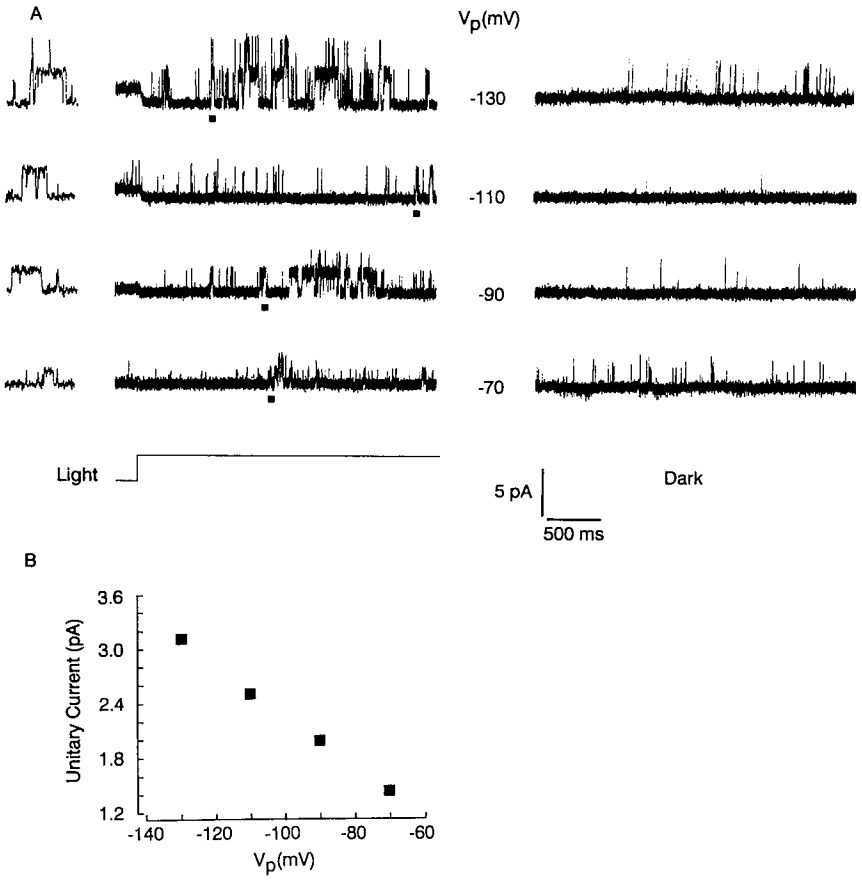

FIGURE 7. Light activated channels in *Pecten* hyperpolarizing photoreceptors. Cell-attached recordings performed in the region where the ciliary appendages are found. A sustained step of light ($4.2 \cdot 10^{15}$ photons·cm^{-2}·s^{-1}) was delivered every 2 min while the pipette potential was maintained at each of the indicated values. Photostimulation activated at least two channels. Portions of the recordings marked by the short thick bars below each trace are expanded on the left to illustrate the appearance of light-dependent unitary currents. In the absence of photostimulation (*right*), channel activity was minimal, and consisted of rare, rapid transitions. (*B*) Single-channel current amplitude as a function of applied pipette potential. The unitary conductance was 27 pS.

recorded from a patch stimulated with a sustained step of light; steady depolarizations to various potentials were applied with the aim of maximizing the driving force and hence the visibility of outward channels (because during the light response the cell hyperpolarizes substantially, approaching E_k). Distinct openings of at least two

channels can be clearly seen after light onset, together with a small, negative-going shunt current due to the cell's hyperpolarizing photoresponse. In the dark, channel activity is minimal in the same range of applied potentials, as demonstrated by the traces on the right, where only sporadic transitions can be observed. These rapid events, which are not fully resolved at the recording bandwidth, may either reflect spontaneous openings of the light-sensitive channels, or the activation of a different type of channel. In part B the amplitude of the unitary current elicited by light is

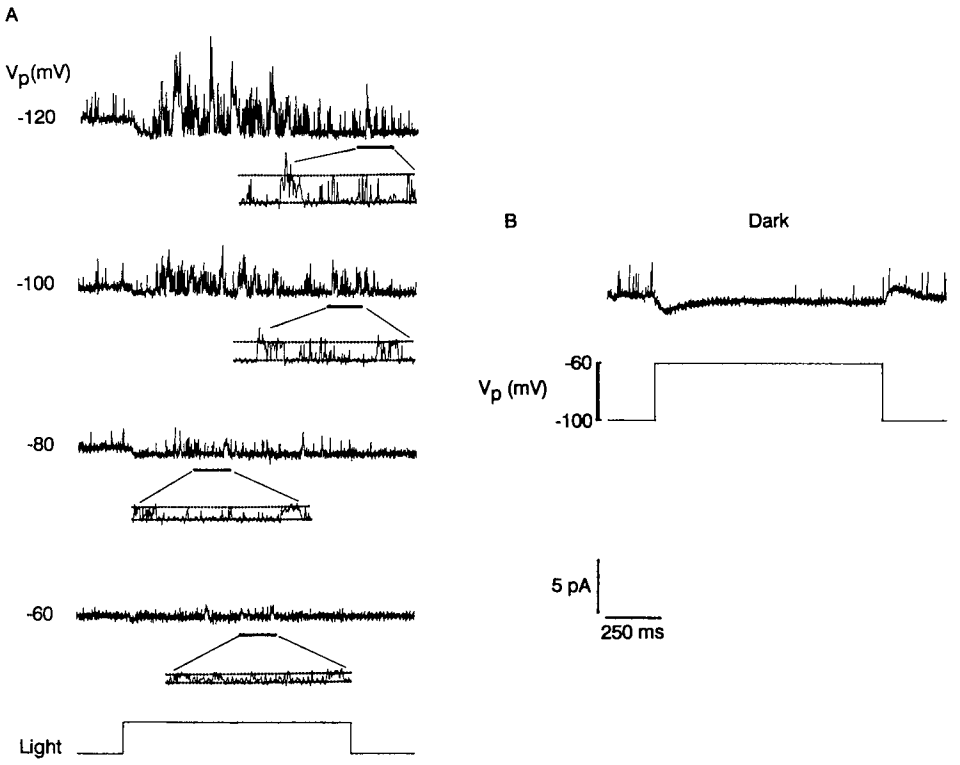

FIGURE 8. Lack of effect of passive hyperpolarization on a patch containing light-dependent channels. (*A*) Effect of stimulation with 1-s steps of light of constant intensity ($5 \cdot 10^{15}$ photons·cm^{-2}·s^{-1}) at different pipette potentials. (*B*) Effect of a 40-mV hyperpolarizing step administered in the dark from a holding pipette potential of −100 mV. The voltage step did not evoke any channel openings, and tended to suppress background activity.

plotted as a function of pipette potential. The resulting relation is nearly linear, with a slope of ≈27 pS (mean 26.2 pS ± 0.97 SD, n = 4). Patch depolarization alone cannot account for the activity observed after light onset; moreover, we can rule out activation by other uncontrolled voltage changes: the experiment shown in Fig. 8 was designed to examine the possibility that passive reduction of the applied depolarization (as occurs during the receptor potential) may somehow be sufficient to bring about channel activation. The recordings shown in Fig. 8 *A* were obtained from

another patch stimulated with 1-s light steps at different pipette voltages. Prominent channel activity is visible after light onset. In Fig. 8 *B* the patch was kept depolarized by 100 mV in the dark and then the depolarization was reduced step-wise by 40 mV for 1 s, mimicking the potential change resulting from maximal photoactivation (e.g., McReynolds and Gorman, 1970*a*). No openings are elicited by the voltage change. Similar observations were obtained in two other patches.

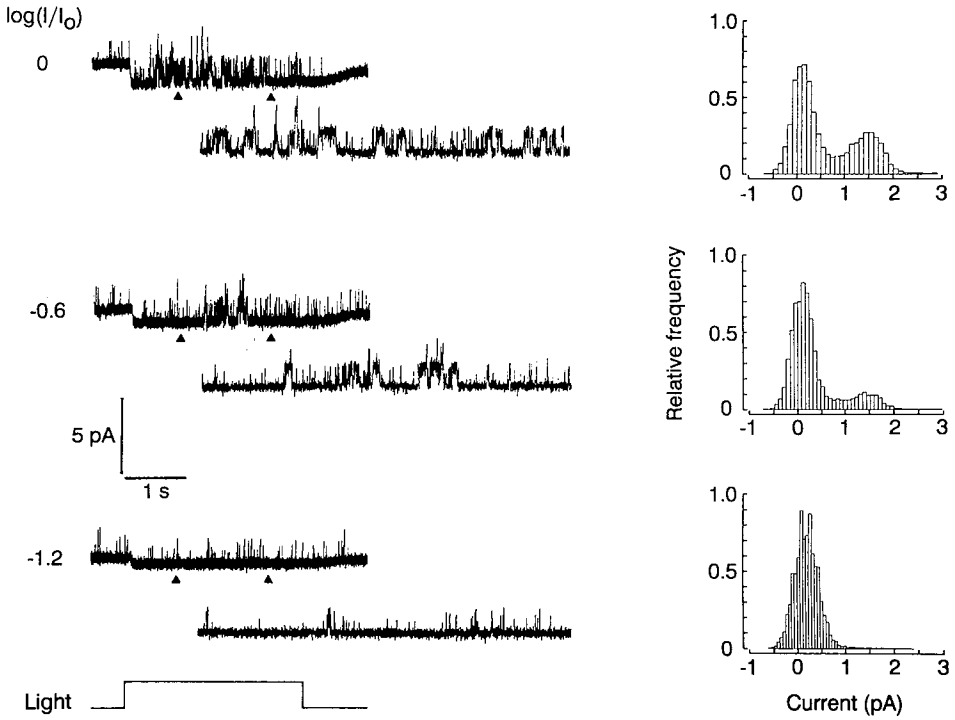

FIGURE 9. Effect of light intensity on channel activation. The pipette potential was held at −90 mV, and 3-s steps of light were attenuated by the values indicated at the left of each trace. Segments of recording during photostimulation (1,500-ms long) marked by the arrowheads are shown on a time scale expanded 4 times to better illustrate the increase in channel openings with higher light intensity. The current-amplitude histograms on the right show a progressively greater dwelling of the channels in the open state, as the stimulus intensity was increased. Unattenuated light intensity $1.5 \cdot 10^{16}$ photons·cm^{-2}·s^{-1}.

Fig. 9 shows that the activity of light-sensitive channels is graded with stimulus intensity: a patch from another cell was depolarized by 90 mV and 3-s steps of light with different attenuation were presented, eliciting outward unitary currents. The fractional open time (F_o) was calculated by setting a threshold half-way between the closed- and the open-state peaks of the current-amplitude histograms. F_o during the light was monotonically related to the intensity of photostimulation (F_o = 0.06 at −1.2 log, 0.14 at −0.6 log, and 0.27 at 0 log). The mean open times, by contrast, did not vary systematically as a function of light intensity.

A prominent feature of hyperpolarizing photoresponses is the post-stimulus rebound excitation which was described in the intact retina of both *Pecten* (McReynolds and Gorman, 1970*a*) and *Lima* (Cornwall and Gorman, 1983), and which is believed to trigger the "off" discharge of action potentials in the distal branch of the optic nerve. This behavior could also be demonstrated in enzymatically isolated ciliary cells, taking advantage of patches containing only depolarization-activated channels. Fig. 10 *A* shows several recordings obtained at different pipette potentials

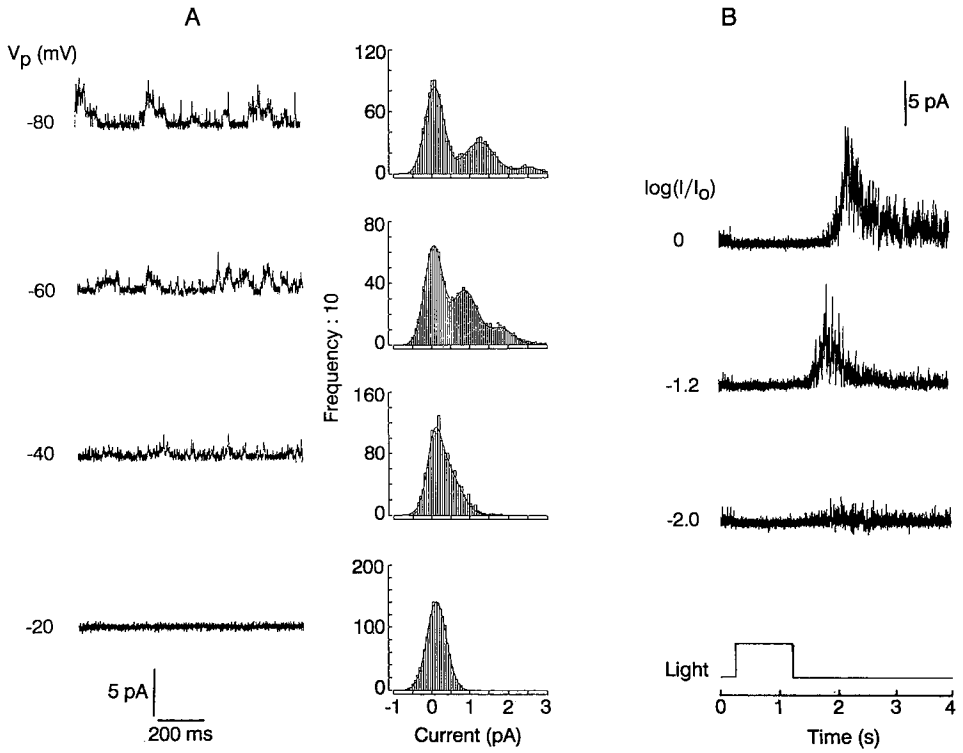

FIGURE 10. (*A*) Depolarization-induced channel activity in a patch with no light-sensitive channels. The patch was quiet at $V_p = -20$ mV, but outwardly directed openings appeared at more depolarized potentials. The histograms by the side of each trace represent current amplitude distributions and were least-squares fitted with Gaussian curves. (*B*) The patch was depolarized by 40 mV and the resulting channel activity (first 200 ms of recording) disappeared after the onset of photostimulation, indicating that the cell hyperpolarized in response to light. A prominent "off" rebound occurred after the termination of the light, graded with the intensity of the preceding stimulus. Unattenuated light intensity $4.2 \cdot 10^{15}$ photons·cm^{-2}·s^{-1}.

from a patch of a *Pecten* photoreceptor; the patch contained no light-sensitive channels, but displayed outward unitary currents as the potential was depolarized. At least two channels were active. The channel's conductance, estimated by fitting a double Gaussian function to the current-amplitude histograms (shown next to each

trace), was 23.5 pS. The pipette potential was then set at −40 mV, causing sustained activation. In Fig. 10 *B*, presentation of a step of light resulted in immediate suppression of depolarization-induced channel activity, reflecting the fact that the cell hyperpolarized in response to light. After the termination of the light a prominent rebound occurred, clearly reminiscent of the "off" response. The amplitude of the rebound response was directly related to the intensity of the preceding light stimulus.

DISCUSSION

Hyperpolarizing photoreceptors, long believed to coexist with depolarizing cells in the eye of the fringe clam, *Lima scabra,* and the bay scallop, *Pecten irradians,* were isolated enzymatically, and their electrophysiological properties were examined using the tight-seal technique. Previous electrical recordings in these organisms had been made in whole retinas, without subsequent identification of the cells studied. Our measurements in solitary cells are consistent with all the salient features reported in intact tissue, leaving no doubt that hyperpolarizing responses to light are indeed primary receptor potentials, that arise from a distinct category of photoreceptors. In addition, we obtained cell-attached patch-electrode recordings from *Pecten* photoreceptors and examined the properties of single ionic channels activated by light.

Previous attempts to identify isolated ciliary photoreceptors were unsuccessful, despite the fact that these cells are presumably represented in numbers comparable to the rhabdomeric cells, at least in *Pecten* (Dakin, 1910). Several factors appear to have adversely affected earlier attempts: first, enzymatic conditions that are near-optimal for dispersing rhabdomeric photoreceptors (Nasi, 1991*a;* Nasi and Gomez, 1992) appear to be less effective on ciliary cells, perhaps owing to a different composition of the retinal matrix surrounding them. A second factor was the lack of easily discernible anatomical features, such as the conspicuous villous lobe of rhabdomeric cells. In *Lima,* for example, the cilia of hyperpolarizing cells are not easily resolved in bright-field microscopy, making difficult to distinguish them from glia and other unpigmented cells. Third, ciliary photoreceptors (especially from *Lima*) proved quite resilient to plating, and, as a consequence, a large fraction was inevitably washed away when the flow chamber was superfused. The ones that remained were often precariously attached, so that great care was needed to avoid detaching them with the recording electrode.

The properties of the photocurrent of isolated ciliary receptors under whole-cell clamp are consistent with previous reports using intracellular measurements in situ and display striking similarities between the two species. Light stimulation elicits an outward current that is graded with light intensity, and is accompanied by a decrease in the input resistance of the cell. The reversal potential of the photocurrent is close to E_K, and shifts with changes in $[K]_o$ in a way closely predicted by the Nernst equation, implying a rather high selectivity for potassium ions (Gorman and McReynolds, 1978). A salient feature of ciliary photoreceptors is the increase of the light-sensitive conductance with membrane depolarization. Outward rectification has also been reported in amphibian rods (Yau and Baylor, 1989) and also in *Limulus* rhabdomeric photoreceptors with large depolarizations (Bacigalupo, Chinn, and

Lisman, 1986). It is noteworthy that in ciliary photoreceptors the outward rectification is prominent in the normal operating range of voltages (namely, −30 to −70 mV); as a consequence, this phenomenon is likely to be of physiological significance (for example, as a negative feedback mechanism that reduces the photocurrent when the cell generates a hyperpolarizing receptor potential). Like in vertebrate rods, removal of divalent cations from the extracellular solution greatly reduces the rectification in *Pecten* cells, suggesting that a major underlying mechanism is voltage-dependent blockage of the light-sensitive channels by Ca^{2+} and Mg^{2+}.

In *Pecten*, direct measurements of single-channel currents activated by light could be performed in cell-attached patches. These recordings were presumably made possible by the favorable geometry of the ciliary appendages, which is less formidable than in *Lima*. The unitary conductance of light-sensitive channels is significantly smaller than that of the primary light-sensitive channels of depolarizing receptors (Bacigalupo and Lisman, 1983; Nagy and Stieve, 1990; Nasi and Gomez, 1992), and is similar to the conductance of light-suppressed single-channel currents in amphibian rods, measured in the absence of divalent cations (Matthews and Watanabe, 1987).

There is presently no information about the transduction chain in hyperpolarizing invertebrate visual cells. Some clues on the gating of a potassium-selective light-sensitive conductance, however, have recently been obtained from a different system, an extraocular photoreceptor found in the abdominal ganglion of the opisthobranch mollusc *Onchidium verrunculatum*. In that cell, light evokes a slow depolarizing response due to the closing of a class of potassium channels (Gotow, 1989); injection of cGMP elicits an outward current which is similar to the dark current and is also light-suppressible (Nishi and Gotow, 1989; Gotow and Nishi, 1991). These observations set a precedent for guanine cyclic nucleotide involvement in the control of a light-controlled K channel, similar in many ways to the transduction process of vertebrate photoreceptors. On the other hand, in view of the widespread presence of Ca-activated K channels in many cell types and the generality of photo-induced cytosolic Ca transients in invertebrate photoreceptors (Brown and Blinks, 1974), it is also possible that calcium ions could be implicated as second messengers in the hyperpolarizing visual cells of *Lima* and *Pecten*.

Ciliary photoreceptors appear to be relatively widespread among mollusks, and their hyperpolarizing photoresponse is thought to subserve an important biological function: by triggering a discharge of action potentials at the termination of illumination (Hartline, 1938; Mpitsos, 1973; Cornwall and Gorman 1979, 1983), these cells initiate defensive reflexes in response to dark objects and shadows appearing in the visual field of the animal ("shadow reflex"). It had been previously pointed out that the presence of cilia vs microvilli establishes a division between the two major lines of photoreceptor cells found across the animal kingdom (Eakin, 1965), which represent two evolutionary solutions to the problem of optimizing the packing of a large number of membrane-imbedded photopigment molecules. It is remarkable that not only both classes of photoreceptors coexist in the same eye, but also that the same dual system is found across organisms belonging to different superfamilies such as *Lima* and *Pecten*.

We are grateful to Drs. Joel Brown and John Lisman for carefully reading and criticizing an earlier version of the manuscript, and to the staff of the Marine Biological Laboratory, where the experiments on *Pecten* were performed.

Supported by NIH grant RO1 EY07559.

Original version received 16 August 1993 and accepted version received 8 December 1993.

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