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IONIC MECHANISMS OF PHOTOTRANSDUCTION IN PHOTORECEPTOR CELLS FROM THE EPISTELLAR BODY OF THE OCTOPUS *ELEDONE CIRRHOSA*

CHRISTOPHER S. COBB[1] and RODDY WILLIAMSON[1,2]

[1]*The Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK* and
[2]*Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK*

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Summary

Intracellular recordings were made from extraocular photoreceptor cells within isolated epistellar bodies of the lesser or northern octopus *Eledone cirrhosa*. The cells had resting potentials around −41±5 mV (mean ± S.D., *N*=60) and showed light-flash-induced membrane depolarisation. The evoked response to a brief light flash consisted of a transient peak depolarisation, followed by a plateau component. The magnitude of the light-induced peak depolarisation response was decreased by bathing the epistellar body in artificial sea water (ASW) low in Na^+, where $choline^+$ replaced Na^+, or by passing steady depolarising current. Replacement of external Na^+ by Li^+ had no effect on the light-stimulated response. The external application of the Na^+ channel blocker tetrodotoxin (3 μmol l^{-1}) increased the light-evoked response, but this was accompanied by a loss of action potential activity. The amplitude and duration of the response to a light flash was increased by bathing the epistellar body in ASW low in Ca^{2+}, or in ASW containing 10 mmol l^{-1} Co^{2+}, and after intracellular microinjection of the Ca^{2+} buffer EGTA. Intracellular microinjection of Ca^{2+} or inositol 1,4,5-trisphosphate, or external application of the phospholipase C inhibitor U-73122, had no apparent effect on the light-evoked response. These results are consistent with the interpretation that (1) the majority of the light-induced inward current is carried by Na^+, probably *via* a non-selective cation channel, and (2) an increase in the intracellular free Ca^{2+} concentration, mediated by the phototransduction process, is involved in regulating the light-induced inward photocurrent and thus, in effect, determines the amplitude, time course and sensitivity of the receptor potential.

Key words: ions, phosphoinositide signalling, U-73122, phototransduction, electrophysiology, extraocular photoreceptor, epistellar body, Cephalopoda, octopus, *Eledone cirrhosa*.

Introduction

Progress in understanding the biochemical and physiological mechanisms underlying invertebrate phototransduction has been hindered by a dearth of suitable experimental model systems. For example, although extensive physiological data have been obtained from the eyes of *Limulus polyphemus* (Bacigalupo and Lisman, 1983; Brown et al., 1984), and electrophysiological recordings from the eyes of wild-type and various mutants of *Drosophila melanogaster* (Bloomquist et al., 1988; Hardie and Minke, 1993) have been very informative, such preparations have been less useful for biochemical studies because of the minute amounts of tissue involved and its heterogeneity. In contrast, the eyes of cephalopods yield large amounts of retinal tissue, almost exclusively consisting of photoreceptor cells (75–90 % of total retinal volume; Adams and Hagins, 1960), and the apical phototransducing rhabdomeric layer of the retina can be detached in almost pure form (Baer and Saibil, 1988). Because of these advantages, the biochemistry of phototransduction in cephalopods has been extensively investigated (Baer and Saibil, 1988; Saibil, 1984; Szuts et al., 1986; Brown et al., 1987). Unfortunately, the corresponding electrophysiological aspects of phototransduction in cephalopod retinal photoreceptor cells have proved more intractable to study because these photoreceptors are long and very thin, and so intracellular recording or patch-clamp work is technically challenging (Pinto and Brown, 1977; Nasi and Gomez, 1992). However, most cephalopods also have groups of extraretinal photoreceptors (Mauro, 1977; Budelmann et al., 1997) and, as shown in the present study, these may provide the physiological data needed to complement information obtained from the biochemical and molecular investigations.

The extraocular photoreceptors of octopods, such as *Eledone moschata* and *Eledone cirrhosa*, are located inside the mantle sac and appear as a small (<1 mm) pigmented vesicle on the ventral posterior margin of both stellate ganglia (Bauer, 1909; Young, 1936; Mauro, 1977; Cobb and Williamson, 1998); these pigmented vesicles were termed the ‘epistellar bodies’ (Young, 1929, 1936). The ultrastructure of the epistellar body wall from *Eledone moschata* and *Octopus bimaculatus* has been shown to consist of packed arrays of photoreceptor cells between epithelial (supporting) cells and pigment cells (Perrelet and Mauro, 1972; Budelmann et al., 1997). These

photoreceptor cells send processes with a parallel arrangement of microvilli reminiscent of retinal rhabdomeres into the lumen of the epistellar body (Nishioka et al., 1962; Perrelet and Mauro, 1972) and, in *Eledone moschata*, 'rhabdomeric' fractions from the epistellar body have an absorption spectrum characteristic of rhodopsin (Nishioka et al., 1966; Mauro, 1977). The epistellar body photoreceptor cells have been shown to give a depolarising response to light, often accompanied by a burst of action potentials, (Mauro and Baumann, 1968; Mauro, 1977; Cobb and Williamson, 1996, 1998), and these responses are similar to those obtained from cephalopod retinal photoreceptors (Hagins et al., 1962; Pinto and Brown, 1977; Tomita, 1968).

The present study uses electrophysiological techniques to examine ionic and phosphoinositol signalling during phototransduction in the extraocular photoreceptor system of the octopus *Eledone cirrhosa*.

Materials and methods

Collection and maintenance of experimental animals

Lesser or northern octopus, *Eledone cirrhosa* Lamarck, 1798 (dorsal mantle length 5–14 cm), of both sexes were used in this study. Octopus were caught offshore near the coast of Plymouth, UK, at a depth of 10–15 m during short-haul trawls and kept in laboratory holding tanks with flow-through, filtered ($2\,l\,min^{-1}$) and cooled (12–18 °C) sea water from a closed circulation system until required. Escape of octopus from the tank was prevented by lining the tank walls with 1 cm thick sheet plastic foam (Boyle, 1981). Octopus were supplied with live crabs (*Carcinus maenus*) *ad libitum*.

Perfused chemical solutions

Artificial sea water (ASW) contained $470\,mmol\,l^{-1}$ NaCl, $10\,mmol\,l^{-1}$ KCl, $50\,mmol\,l^{-1}$ $MgCl_2$, $10\,mmol\,l^{-1}$ $CaCl_2$ and $10\,mmol\,l^{-1}$ Mops (Sigma, UK). pH was adjusted to 7.8 with $1\,mol\,l^{-1}$ NaOH, and osmolarity was maintained at 1010 mosmol kg^{-1}. The composition of choline ASW was the same as that of ASW except that equimolar choline chloride was substituted for NaCl, and Mops by Hepes (Sigma, UK). Li^+ ASW contained an equimolar replacement of NaCl by LiCl. Nominally Ca^{2+}-free ASW contained $470\,mmol\,l^{-1}$ NaCl, $10\,mmol\,l^{-1}$ KCl, $60\,mmol\,l^{-1}$ $MgCl_2$ and $10\,mmol\,l^{-1}$ Hepes, and 0-Ca^{2+} ASW contained $470\,mmol\,l^{-1}$ NaCl, $10\,mmol\,l^{-1}$ KCl, $60\,mmol\,l^{-1}$ $MgCl_2$, $10\,mmol\,l^{-1}$ Mops and $6\,mmol\,l^{-1}$ EGTA (Sigma, UK). The phospholipase C (PLC) inhibitor U-73122 (1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)-amino)-hexyl]-1H-pyrrole-2,5-dione; Calbiochem-Novabiochem Ltd, UK] was dissolved in dimethylsulphoxide (DMSO; Sigma, UK) to make up a $2\,mmol\,l^{-1}$ stock solution and was then diluted with ASW to make up final concentrations.

Intracellular recordings

For intracellular recordings, octopus were killed by decapitation after anaesthesia in 3 % ethanol in sea water, and the right and left stellate ganglia and attached epistellar bodies were dissected from the dorsal mantle wall of each animal. The epistellar body and attached stellate ganglion were washed in fresh sea water and pinned out ventral side up in a Sylgard-lined recording dish (volume 6 ml) filled with filtered ASW. All recordings were made at room temperature, i.e. between 18 and 23 °C. During some experiments, the composition of the solutions surrounding the preparation was changed by perfusion at a rate of $12\,ml\,min^{-1}$ from ASW to choline ASW, Li^+ ASW, nominally Ca^{2+}-free ASW, 0-Ca^{2+} ASW or ASW containing $10\,mmol\,l^{-1}$ $CoCl_2$ or $10\,\mu mol\,l^{-1}$ and $100\,\mu mol\,l^{-1}$ U-73122, depending on the experiment. The pharmacological agent tetrodotoxin (TTX, Sigma, UK) was pipetted in a small volume of ASW into the recording dish when required.

Illumination stimuli were provided by a Schott KL1500 cold light source with a quartz halogen bulb (Thorn EMI; 15 V, 150 W). The light was passed through a standard heat filter into a glass-fibre light guide and then, *via* an electronically controlled shutter and a second fibre light guide, to illuminate the ventral side of the stellate ganglion and the epistellar body. The duration of the light flash was set by the electronic shutter (Uniblitz TI32; Optilas Ltd, UK) to between 200 and 500 ms. The light intensity was varied by inserting neutral density filters into the light path and was measured at the level of the preparation using a portable radiometer or photometer (Ealing Electro-Optics, UK; model 27-5479). An additional uncalibrated photocell was used to record the precise timing and duration of the stimulus light flashes.

Intracellular recordings from photoreceptor cells in the epistellar body wall were obtained using microelectrodes made of borosilicate glass capillaries, with inner filaments (Clark Electrochemical, UK; GC-150F, 1.5 mm o.d., 0.86 i.d.), filled with $4\,mol\,l^{-1}$ potassium aspartate, adjusted to pH 7.8 with KOH. These microelectrodes had tip resistances exceeding 40 MΩ when measured in ASW. A conventional bridge circuit was used (AxoClamp 2B amplifier; Axon Instruments, Inc, USA) for recording resting, generator and receptor potentials and for passing current pulses through the microelectrode. In some experiments, photoreceptor cells were injected with the Ca^{2+} buffer EGTA by impaling them with a microelectrode containing $1\,mol\,l^{-1}$ EGTA, adjusted to pH 7.8 with KOH, and using injections of continuous hyperpolarising current (–3.0 nA for 3 s). The resting potential was recorded during electrode insertion and in response to light flashes, before and after switching to current injection. Microinjection of Ca^{2+} was also attempted using various electrode solutions containing Ca^{2+}, e.g. $0.1\,mol\,l^{-1}$ to $1\,mol\,l^{-1}$ $CaCl_2$ solutions, buffered or not, some containing an additional electrolyte to lower resistance and improve the passage of current; none of these was successful, all blocking under current injection probably because of the resulting high resistances of the electrodes and the Ca^{2+} forming molecular complexes at the electrode tip. In some experiments, a $1\,mmol\,l^{-1}$ solution of inositol 1,4,5-trisphosphate ($InsP_3$; Sigma, UK) made up in $100\,mmol\,l^{-1}$ potassium aspartate (D-aspartic acid; Sigma, UK; adjusted to pH 7.8 with KOH) was ejected from the microelectrode by applying brief pressure pulses to the back of the microelectrode

or by brief current injection. As before, the cell resting potential was recorded during electrode insertion, and receptor potentials were recorded in response to light flashes before and after pressure or current injection.

The signal from the microelectrode was amplified and, together with the signal from the photocell monitor, was passed to a computer-controlled signal averager (CED 1401 computer interface running Sigavg software; Cambridge Electronic Design, UK). In some experiments, responses were averaged to improve signal-to-noise ratios; stimuli were separated in most experiments by at least 20 s to avoid any decrement in the photoresponse due to adaptation (Cobb and Williamson, 1998). Illumination of the experimental 'darkroom' was also provided by a dim-red safelight (240 V, 100 W), which was found not to compromise the photoresponse. The stellate ganglion and epistellar body preparation remained viable for well over 6 h and was dark-adapted under red safelight (Kodak 1A; >650 nm) before starting experiments. The preparation did not require constant perfusion of ASW to remain viable; however, during the course of an experiment, the preparation was perfused with ASW between recordings. In some cases, the preparation was left at 4 °C in ASW overnight and recordings were made the following day, with individual intracellular recordings being stable in some cases for over 1 h. No 'quantum bumps' were recorded from the epistellar photoreceptor cells of *Eledone cirrhosa* during this study or by Cobb and Williamson (1998). However, 'quantum bumps' have been observed during intracellular recordings from epistellar body photoreceptor cells in *Eledone moschata* (Mauro and Baumann, 1968).

Results

The stellate ganglion and epistellar body of the octopus *Eledone cirrhosa* are shown in Fig. 1. Single-cell, intracellular impalements of the epistellar body photoreceptor cells revealed that they had membrane resting potentials around −41±5 mV (mean ± S.D., *N*=60) and displayed a low frequency of spontaneous action potentials. The action potentials were between 5 and 25 mV in amplitude and were therefore probably not actively conducted into the cell soma. The recorded resting potentials were lower in some photoreceptor cells than the range −42 to −56 mV reported by Cobb and Williamson (1998), perhaps indicating photoreceptor cell damage in some cases. A brief flash of white light induced a short depolarisation of the cell (Fig. 2A) with a correlated increase in action potential firing frequency. The amplitude of the evoked receptor potential increased with increasing light flash intensity (Fig. 2B), and a plot of stimulus intensity against receptor potential amplitude (Fig. 2C) shows that the amplitude of the evoked receptor potential was linearly correlated with the stimulus light intensity across the range of light stimuli examined in this study.

Ionic mechanisms for the receptor potential

Previous studies have suggested that the receptor potential

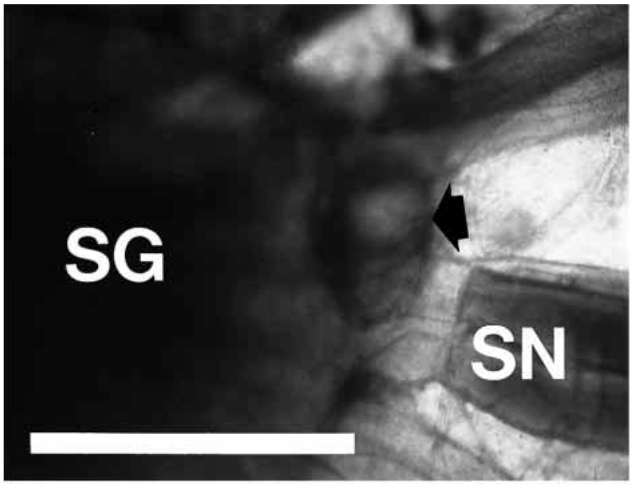

Fig. 1. The stellate ganglion of *Eledone cirrhosa*. The epistellar body (arrow) lies at the posterior end of the stellate ganglion (SG) between stellate nerves (SN). Scale bar, 1 mm.

in the retinal photoreceptor cells of cephalopods and of other invertebrates is generated by a light-induced increase in membrane conductance, carried predominantly by Na^+ (Hagins, 1972; Pinto and Brown, 1977). Photoreceptor cells from the epistellar body of the octopus *Eledone cirrhosa* also show an increase in membrane conductance during light-induced depolarisations, as demonstrated by the reduction in the voltage step produced by a series of constant-amplitude current pulses applied across the cell membrane during a light stimulus (Fig. 3). The reduction in the voltage deflection implies a light-evoked increase in membrane conductance.

If the ions carrying the current during this conductance change are predominantly Na^+, then changing the electrochemical gradient for Na^+ across the photoreceptor cell membrane should change the amplitude of the receptor potential in response to light flashes. One way of achieving this is to alter the cell membrane potential by injecting positive or negative current into the cell through the microelectrode. As shown in Fig. 4A,B, depolarising the cell resulted in a decrease in the light-induced response, whereas hyperpolarising the cell increased the light-induced response. Another way of changing the electrochemical gradient for Na^+ is to remove Na^+ from the external medium by perfusing the system with ASW in which the Na^+ has been replaced by an equimolar concentration of $choline^+$. As seen in Fig. 5A, under these conditions, the light-evoked depolarisation was substantially reduced by 75 % compared with the control value in normal ASW; however, the depolarisation was not entirely abolished. Subsequent perfusion with normal ASW, containing Na^+, resulted in a partial recovery of the receptor potential amplitude. Substituting Li^+ for Na^+ in the ASW produced very little change in the response to light flashes (Fig. 5B), but under these conditions no action potentials were observed, either spontaneous or light-evoked. The majority of voltage-activated Na^+ channels, in both vertebrates and invertebrates, can be blocked by the application of the pufferfish toxin tetrodotoxin

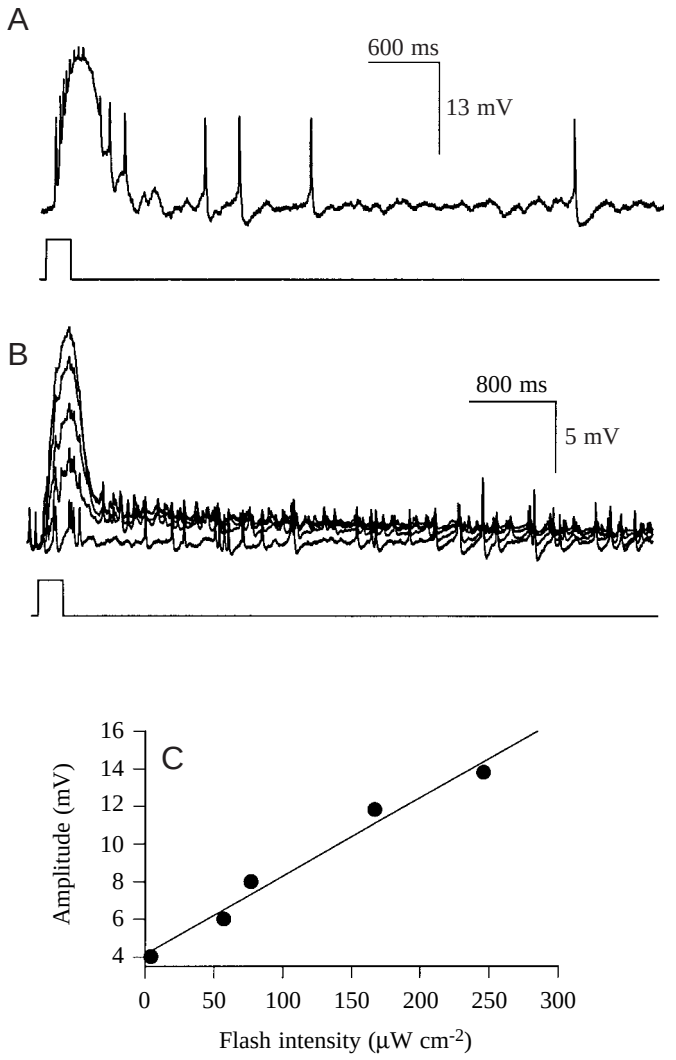

Fig. 2. Intracellular receptor potentials recorded in photoreceptor cells from the epistellar body in response to changes in light stimulus intensity. (A) The upper trace shows a typical depolarisation and associated action potentials from an epistellar body photoreceptor cell evoked by a short-duration, high-intensity light flash stimulus (intensity 246 μW cm^{-2}), as indicated in the lower trace. The photoreceptor cell resting potential was −43 mV. (B) The upper traces show the receptor potentials recorded intracellularly in a single photoreceptor cell in response to a series of light flashes of 200 ms duration and increasing intensity, as indicated in the lower trace. The resting potential was −41 mV. (C) Graph of light flash intensity against receptor potential amplitude responses from B. The line is a best-fit regression line (y=4.13+0.04x, r^2=0.97, P>0.05).

(TTX). When TTX was added to the external ASW solution (Fig. 5C), an increase in the light-evoked response was observed, again accompanied by a loss of action potential activity.

Effects of external Ca^{2+} concentration on the receptor potential

Reducing the concentration of external Ca^{2+}, by perfusion with ASW that was nominally free of Ca^{2+}, but which

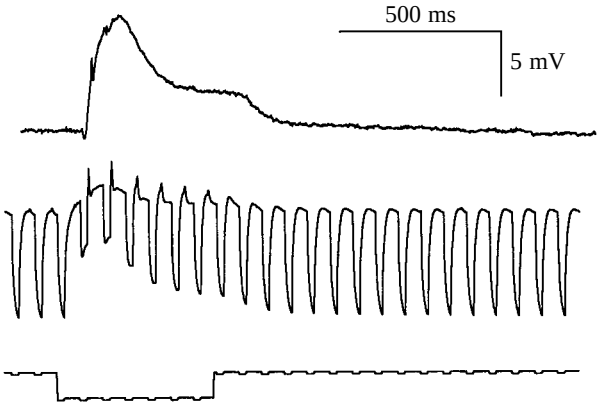

Fig. 3. An increase in photoreceptor cell membrane conductance is associated with the depolarising receptor potential. The top trace shows a light-evoked receptor potential recorded from a cell with a resting potential of −44 mV in response to a 500 ms light flash of 167 μW cm^{-2} intensity before current pulses were passed through the microelectrode into the photoreceptor cell. The middle trace shows the membrane response of the photoreceptor cell to the repetitive injection of constant current pulses of 0.4 nA during the depolarisation shown in the top trace. The decrease in the resultant voltage drop across the membrane indicates an increase in membrane conductance. The bottom trace shows the timing of the current pulses superimposed on the marker for the light flash stimuli.

nevertheless did contain trace amounts of Ca^{2+}, resulted in a significant alteration in the responses of the photoreceptor cells to light flashes of constant intensity and duration. Fig. 6A shows that under these conditions there was an increase in the amplitude and duration of the light-evoked response. Subsequent perfusion with normal ASW, containing Ca^{2+}, reduced the evoked receptor potential response to a level close to that of the control. Further elimination of external Ca^{2+}, by perfusion of the photoreceptor cells with 0-Ca^{2+} ASW, which contained the Ca^{2+} buffer EGTA, resulted in a further increase in the amplitude and duration of the light-evoked response (Fig. 6B) compared with that obtained in the low-Ca^{2+} medium (nominally Ca^{2+}-free ASW). However, in this case, perfusion with normal ASW containing Ca^{2+} caused the receptor potential to collapse. As well as the changes in the light-evoked receptor potential responses, both the low- and 0-Ca^{2+} solutions also abolished all action potential activity in the photoreceptor cells, both spontaneous activity and evoked activity (Fig. 6A,B). Since reducing the external Ca^{2+} concentration resulted in an increase in the amplitude and duration of the light-evoked photoreceptor cell response, it may be that an influx of Ca^{2+} is involved in the termination of the light-induced receptor potential. To investigate this further, the Ca^{2+} channel blocker Co^{2+} was added to the normal external ASW solution, and light stimuli were applied. Under these conditions, there was an initial increase in both the amplitude and duration of the light-evoked response (Fig. 7) when compared with the control values (normal ASW). However, the responses then decayed over a period of 10 min and were not recoverable by washing in fresh ASW (Fig. 7).

Effects of internal Ca^{2+} concentration on the receptor potential

If an influx of Ca^{2+} is involved in the termination of the light-evoked response, then it may be that this can be investigated by directly manipulating the intracellular Ca^{2+} concentration through the intracellular injection of a Ca^{2+} buffer, which will reduce the free ion concentration, or injecting Ca^{2+} itself, which may increase the free ion concentration. It was found that injection of the Ca^{2+} buffer K-EGTA (pH 7) had the effect of initially increasing both the amplitude and the duration of the light-evoked response (Fig. 8). However, over a 10 min period, the response then gradually declined (Fig. 8).

Direct injections of Ca^{2+} from the microelectrode were also

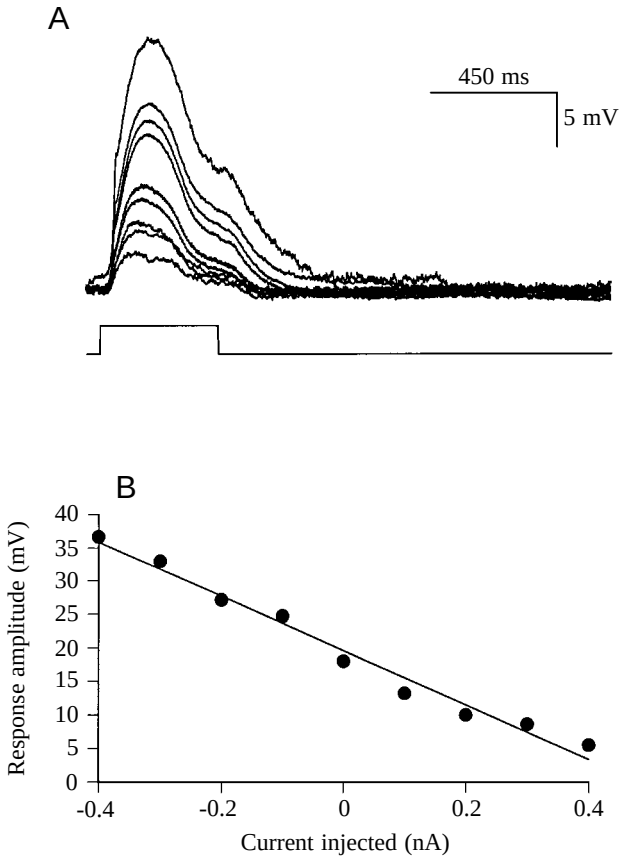

Fig. 4. Decreased and increased electrochemical gradients for Na^+ attenuate and increase the receptor potential, respectively. (A) Passage of depolarising current pulses through the microelectrode into the photoreceptor cell resulted in attenuation of the response to a constant-intensity (167 μW cm^{-2}) light flash stimulus, while a hyperpolarising current increased the amplitude of the response. The 450 ms light flash and the resultant photoresponses were synchronised within the depolarising or hyperpolarising current pulse (3 s pulse with a 6 s repeat time) with a 200 ms delay between the start of the current pulse and the beginning of the light flash stimulus. The resting potential at the start of recording was −37 mV. (B) Graph of intracellular current injection against receptor potential amplitude from the results shown in A. The line is a best-fit regression line (y=19.64–40.63x, r^2=0.98, P>0.05).

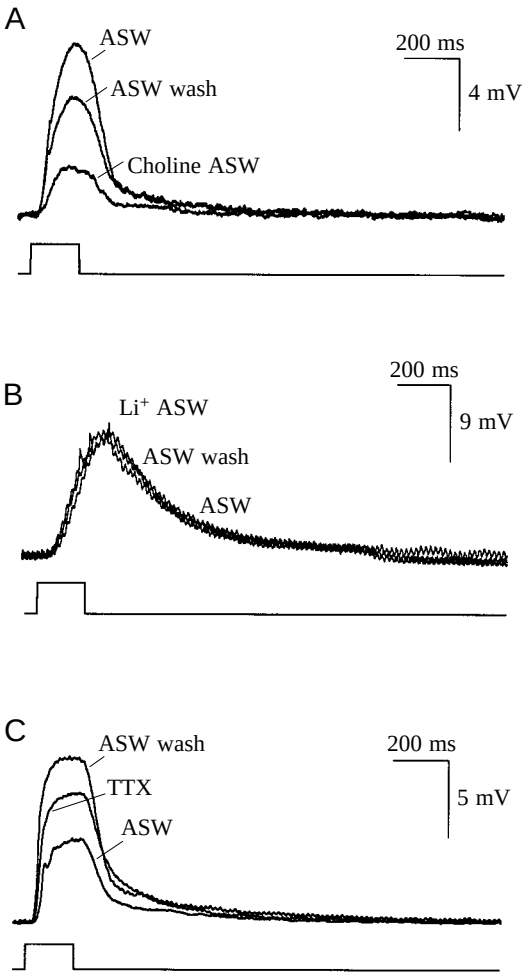

Fig. 5. (A) Decreased electrochemical gradient for Na^+ attenuates the light-induced depolarisation of the photoreceptor cell. The upper traces show the effect of replacing 470 mmol l^{-1} Na^+ with 470 mmol l^{-1} choline$^+$ in the normal artificial sea water (ASW) perfusion solution after 20 min and subsequent reperfusion of the epistellar body preparation with normal ASW after 1 h. Each trace is an average of 10 receptor potentials recorded after the time indicated above. The timing of light flash stimuli (intensity 246 μW cm^{-2}) is indicated in the bottom trace. The resting potential was stable at −38 mV during the experiment. (B) Effect of replacing Na^+ with Li^+ on the light-induced depolarisation. The upper traces show the effect on the light-flash-induced receptor potential of replacement by perfusion of 470 mmol l^{-1} Na^+ by equimolar Li^+ in the ASW after 10 min and subsequent rewashing with ASW containing Na^+ after 30 min. Each trace is an individual receptor potential recorded at the time indicated above. Note the presence of action potentials in ASW only at the start of the experiment. The timing of light flash stimuli (intensity 246 μW cm^{-2}) is indicated in the lower trace. The resting potential was −42 mV during the experiment. (C) Effect of the Na^+ channel blocker tetrodotoxin (TTX) on the light-induced depolarisation of a photoreceptor cell. The upper traces show the effect of bath application of 3 μmol l^{-1} tetrodotoxin on an epistellar body preparation bathed in ASW containing 470 mmol l^{-1} Na^+ after 6 min and the subsequent effect of rewashing with normal ASW after 15 min. Each trace is an average of 10 receptor potentials recorded after the time indicated above. The timing of light flash stimuli (intensity 246 μW cm^{-2}) is indicated in the bottom trace. The resting potential increased from −41 to −33 mV during the experiment.

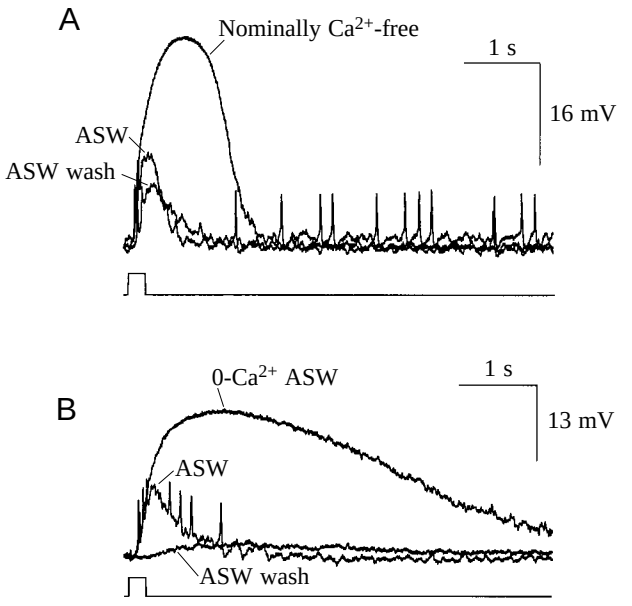

Fig. 6. The amplitude and duration of the light-induced receptor potential are affected by external Ca^{2+}. (A) The upper traces show the effect on the receptor potential of replacement by perfusion of normal ASW containing 10 mmol l^{-1} Ca^{2+} by nominally Ca^{2+}-free artificial sea water (ASW) containing trace levels of Ca^{2+} after 18 min and subsequent rewashing with normal ASW after 1 h. Each trace is an individual receptor potential recorded at the time indicated above. Note the presence of action potentials in ASW only at the start of the experiment. The resting potential was unchanged at −39 mV during this experiment. The timing of the light flash stimuli (intensity 167 μW cm^{-2}) is indicated in the bottom trace. (B) The upper traces show the effect on the receptor potential of replacement by perfusion of normal ASW containing 10 mmol l^{-1} Ca^{2+} ASW by Ca^{2+}-free ASW (0-Ca^{2+} ASW) containing EGTA after 5 min and subsequent rewashing with normal ASW after 1 h. Each trace is an individual receptor potential recorded at the time indicated above. Note the presence of action potentials in ASW only at the start of the experiment. The resting potential was unchanged at −36 mV during the experiment. The timing of the light flash stimuli (intensity 167 μW cm^{-2}) is indicated in the bottom trace.

tried with the intention of raising the intracellular Ca^{2+} concentration. However, although various Ca^{2+}-containing electrode solutions were tried (see Materials and methods), these usually blocked the microelectrode as soon as positive pulses were applied or had no consistent effect on the responses to light flashes. Similarly, direct intracellular injections of inositol 1,4,5-trisphosphate ($InsP_3$) from the microelectrode, using either pressure or current pulses to induce bulk flow effects, failed to have any clear action on the responses evoked by applied light flashes. Finally, the external application of 10 or 100 μmol l^{-1} solutions in ASW of the selective phospholipase C (PLC) inhibitor U-73122, which inhibits the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to $InsP_3$, also failed to have any effect on light-evoked extracellular generator potentials recorded from several epistellar bodies.

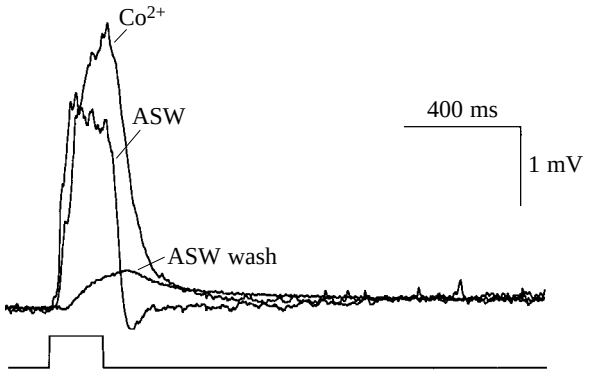

Fig. 7. Effect of the Ca^{2+} channel blocker Co^{2+} on the light-induced receptor potential. The upper traces show the effect of bath application by perfusion of 10 mmol l^{-1} Co^{2+} in artificial sea water (ASW) on the receptor potential after 5 min and subsequent rewashing with Co^{2+}-free ASW after 50 min on the epistellar body preparation, originally bathed in ASW. Each trace is an average of 10 receptor potentials recorded after the time indicated above. The timing of the light flash stimuli (intensity 77 μW cm^{-2}) is shown in the bottom trace. The resting potential was unchanged at −43 mV during this experiment.

Discussion

These results have shown that extraocular photoreceptor cells in the epistellar body of the octopus respond to an increase in illumination with a depolarisation of the cell membrane potential, and that this depolarisation is due to an increase in the cell membrane conductance. The current flowing through the depolarising conductance is mainly carried by Na^+ because the light-evoked response is diminished when the driving force for Na^+ entry is reduced, either by replacing the extracellular Na^+ with choline$^+$ or by reducing the potential gradient for Na^+ entry. Similar illumination-induced increases in Na^+ conductance and intracellular Na^+ concentrations have been observed in cephalopod retinal photoreceptors (e.g. Hagins et al., 1960, 1962, Hagins, 1965; Duncan and Croghan, 1973; Duncan and Weeks, 1973; Pinto and Brown, 1977; Clarke and Duncan, 1978; Takagi et al., 1987; Takagi, 1994c). Other invertebrate photoreceptor cells also show light-stimulated depolarisation as a consequence of increased Na^+ conductance across the cell membrane, including those of the honeybee (Fulpius and Baumann, 1969), barnacle (Brown et al., 1969, 1971) and *Limulus polyphemus* (Millecchia and Mauro, 1969a,b). The receptor potential recorded from extraocular photoreceptor cells in the epistellar body of the octopus was not reduced or abolished by external application of the membrane Na^+ channel blocker TTX, in fact the receptor potential was slightly enhanced. This increase may be due to an increase in the input resistance of the cell, resulting from the blockage of axonal TTX-sensitive Na^+ channels. Similar observations have been made with retinal photoreceptor cells from other cephalopods, such as *Sepiola atlantica* (Duncan and Weeks, 1973), and TTX failed to block the initial component of the lateral eye photoresponse in *Limulus polyphemus*

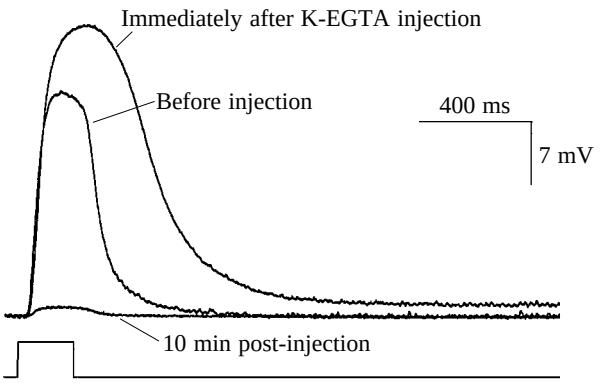

Fig. 8. Injection of K-EGTA into a photoreceptor cell resulted in changes in the amplitude and duration of the receptor potential in a similar way to those observed when the external Ca^{2+} concentration was decreased (Fig. 6). Each upper trace is an average of 10 receptor potentials recorded after the time indicated below. Receptor potentials are shown before intracellular injection of K-EGTA, immediately after K-EGTA injection and after 10 min of perfusion with ASW. The resting potential was unchanged at −41 mV during this experiment. The lower trace shows the timing of light flash stimuli (intensity 167 $\mu W\,cm^{-2}$).

(Dowling, 1968; Wulff and Mendez, 1973), although it should be noted that others have blocked this photoresponse with TTX (Benolken and Russell, 1967).

The present study shows that, in extraocular photoreceptor cells from the epistellar body of octopus, the light-evoked response is not diminished when extracellular Na^+ is replaced by equimolar Li^+. Li^+ is known to inhibit inositol 1,4,5-trisphosphate phosphatase and might therefore have been expected to disrupt any phosphoinositide signalling system present within the extraocular photoreceptor cells (Sakakibara et al., 1994). The failure of Li^+ to affect the light-evoked response in these photoreceptor cells argues against the involvement of $InsP_3$ in the phototransduction process. In experiments using retinal photoreceptor cells from the squid *Loligo pealei*, the sepiolid *Sepiola atlantica* and *Octopus vulgaris*, replacement of the extracellular Na^+ by Li^+, also failed to abolish the receptor potential, possibly as a result of a non-specific ionic increase in membrane conductance (Pinto and Brown, 1977; Duncan and Pynsent, 1979; Takagi, 1994a–c). It seems likely, therefore, that a similar mechanism is present in octopus extraocular photoreceptor cells, which may also explain why replacement of extracellular Na^+ by $choline^+$ reduced the receptor potential by only 75 % during perfusion with choline ASW. In comparison, ionic substitution of extracellular Na^+ with $choline^+$ has been shown reversibly to reduce the receptor potential in cephalopod retinal photoreceptor cells (*Loligo pealei* and *Sepiola atlantica*) by over 90 % (Hagins et al., 1960, 1962; Duncan and Weeks, 1973).

The results described in the present study show that extraocular photoreceptor cells in the octopus epistellar body respond to reduced extracellular Ca^{2+} levels (trace or zero Ca^{2+}) with an increase in the amplitude and duration, and hence sensitivity, of the light-evoked depolarisation. Similar effects were seen after blockage of the photoreceptor cell membrane Ca^{2+} channels with extracellular cobalt, or in response to increased buffering of the free intracellular Ca^{2+} concentration with K-EGTA, again suggesting that free intracellular Ca^{2+} is involved in terminating the light-induced membrane conductance. Studies on cephalopod retinal photoreceptor cells from *Sepiola atlantica* and *Loligo pealei* have shown that, in these cells, reducing the extracellular Ca^{2+} concentration also results in an increase in photocurrent and, by affecting the amplitude and time course of the receptor potential, changes the sensitivity of the photoreceptor cell (Duncan and Weeks, 1973; Weeks and Duncan, 1974; Pinto and Brown, 1977). Photoreceptor cells from other invertebrates, such as *Limulus polyphemus* and *Drosophila melanogaster* (Millecchia and Mauro, 1969b; Lisman and Brown, 1975a,b; Lisman, 1976), respond to decreased extracellular Ca^{2+} concentrations in a similar manner to cephalopod retinal photoreceptors, for example Pinto and Brown (1977) and, in the present study, with octopus extraocular photoreceptor cells. Voltage-clamp experiments with photoreceptor cells from other invertebrates, e.g. *Limulus polyphemus* (Millecchia and Mauro, 1969b; Lisman and Brown, 1975a,b; Brown, 1986), *Balanus amphitrite* (Brown et al., 1970, 1988; Werner et al., 1992) and *Drosophila melanogaster* (Hardie, 1991; Hardie and Minke, 1994), have demonstrated the important roled played by Ca^{2+} in directly regulating the flow of ions through Na^+ and Ca^{2+} exchange channels in the membrane and, thus, its role in determining the amplitude and time course of the receptor potential in invertebrate photoreceptor cells (Minke and Armon, 1984; O'Day and Gray-Keller, 1989). In *Limulus polyphemus* ventral photoreceptor cells and *Calliphora vicina* ommatidia, the decline from the peak of the transient to the plateau depolarisation of the receptor potential has been shown to occur over approximately the same duration as an increase in the free intracellular Ca^{2+} concentration (Brown and Blinks, 1974; Hochstrate and Juse, 1991). This finding further implicates free intracellular Ca^{2+} in the regulation of the membrane conductance for Na^+ in invertebrate photoreceptor cells and, therefore, photoreceptor cell light sensitivity (Lisman and Brown, 1975a,b).

Although the extracellular application of the Ca^{2+} channel blocking agent cobalt (10 $mmol\,l^{-1}$) resulted in an increase in the amplitude and duration of the light-evoked response in octopus extraocular photoreceptor cells, as might be expected if a Ca^{2+} influx is involved in the termination of the evoked response, the response was much smaller than that achieved by reducing the external concentration of Ca^{2+}. In studies of vertebrate photoreceptors, the application of cobalt had little effect on the receptor potential (Dong et al., 1990), and although cobalt application irreversibly changed the amplitude and duration of light-induced receptor potentials in the insect eye (Mojet, 1993), this appeared to be due to intracellular actions of cobalt. Whether, in the present study, the cobalt-induced changes in receptor potential amplitude and duration

were due to toxic actions of intracellular cobalt or simply to extracellular blocking of membrane Ca^{2+} channels remains to be established.

The progress made in identifying the membrane conductances underlying invertebrate phototransduction has not been matched in an identification of the associated intracellular signalling cascade, although various pathways have been proposed, including those involving cyclic nucleotides and phosphoinositides (Nagy, 1993; Gotow and Nishi, 1991). In the phosphoinositide (PI) signalling system, the target enzyme of the GTP-binding protein is phospholipase C (PLC), which acts on the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to form inositol 1,4,5-trisphosphate ($InsP_3$). In support of this view, biochemical assays in squid retina have demonstrated a light-dependent activation of PLC (Baer and Saibil, 1988), as well as rapid $InsP_3$ production after photostimulation (Szuts et al., 1986; Brown et al., 1987). Corresponding electrophysiological evidence from *Limulus polyphemus* has been obtained indicating the involvement of $InsP_3$ (Brown et al., 1984; Fein et al., 1984) and the consequent release of Ca^{2+} from intracellular stores (Brown and Rubin, 1984; Payne et al., 1986). In addition, ommatidia from *norpA* mutant *Drosophila melanogaster*, with reduced phospholipase C (PLC) activity, exhibited defective phototransduction compared with wild-type flies (Bloomquist et al., 1988; Hardie and Minke, 1993). However, similar findings also implicate cGMP in invertebrate phototransduction, since intracellular injection of cGMP in *Limulus polyphemus* cells at some sites has an excitatory effect (Johnson et al., 1986), and light enhances cGMP formation in fractions of the retina extract from the squid *Alloteuthis subulata* (Saibil, 1984); however, this result has recently been called into question in the squids *Loligo pealei* and *Loligo opalescens* and in the horseshoe crab *Limulus polyhemus* (Brown et al., 1992). The failure of direct injections of Ca^{2+} or $InsP_3$ to modulate the light-evoked responses in the present experiments may well be due to a failure to inject sufficient material and therefore cannot be taken as compelling evidence for or against the involvement of the phosphoinositide signalling system in octopus extraocular photoreceptor cells. However, the lack of any modulation of the light-induced extracellular generator potential by the external application of the selective phospholipase C inhibitor U-73122 argues against the involvement of the phosphoinositide system and $InsP_3$ in the phototransduction process, although in type B photoreceptor cells from the nudibranch mollusc *Hermissenda crassicornis*, PIP_2 breakdown by PLC on the cell membrane and the resultant direct activation of Na^+-selective channels has been suggested to trigger the photoresponse, without the involvement of $InsP_3$ (Sakakibara et al., 1994). Although light-evoked PLC activity has been demonstrated in squid retinal photoreceptor cells (Baer and Saibil, 1988), we have no evidence for a role for cyclic nucleotides in octopus extraocular phototransduction. The action of U-73122 in the present study contrasts with studies on the ventral photoreceptor cell preparation in *Limulus polyphemus*, where U-73122 has been shown to block one of three photoresponse components (Nagy and Contzen, 1997), but there is no information on the specificity of U-73122 for cephalopod phospholipase (PLC).

In conclusion, this study indicates that octopus extraocular photoreceptor cells are comparable in their light-induced depolarisation and the underlying ionic phototransduction mechanism with those already reported for cephalopod retinal and other invertebrate rhabdomeric photoreceptor cells. However, the evidence from this study tentatively suggests that a light-induced phosphoinositide system plays no role in extraocular phototransduction in octopus. This contrasts with biochemical evidence from cephalopod retinal photoreceptor cells that argues for the involvement of $InsP_3$ in the phototransduction process (Szuts et al., 1986; Baer and Saibil, 1988), although other work has given more prominence to cGMP as the intracellular messenger in molluscan photoreception (Saibil, 1984; Gotow and Nishi, 1991; Takagi, 1994a). Given the similarities and potential differences, it may be that octopus extraocular photoreceptor cells can provide a useful model for the electrophysiological investigation of phototransduction in cephalopods since, in comparison with retinal photoreceptors, the extraocular photoreceptor cells are easier to expose, survive well in the epistellar body during isolation and, of critical importance for electrophysiology, have a much larger cell soma (50 μm in diameter; Cobb and Williamson, 1998) compared with that of squid retinal photoreceptor cells (4–7 μm; Nasi and Gomez, 1992).

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