J. exp. Biol. **168**, 77–91 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

A NEURAL MECHANISM FOR PROCESSING COLOUR INFORMATION IN MOLLUSCAN EXTRA-OCULAR PHOTORECEPTORS

BY TAKAKO NISHI AND TSUKASA GOTOW*

Department of Physiology, School of Medicine, Kagoshima University, Kagoshima 890, Japan

Accepted 10 March 1992

Summary

This study was conducted to clarify the electrical and spectral properties of the combined photoresponses of two extra-ocular photoreceptor neurones, A-P-1 and Es-1, in the abdominal ganglion of the marine mollusc *Onchidium verruculatum*. The depolarizing receptor potential or inward receptor current of Es-1 appeared to result from a decrease in K^+ conductance, in the same way as in A-P-1. The direct photoresponse of Es-1 had a peak at 580 nm, while A-P-1 had its peak at 490 nm. Under normal conditions, Es-1 was hyperpolarized by blue-green light (490 nm) and was depolarized by yellow light (580 nm), indicating that its responses were wavelength-dependent. Slow inhibitory synaptic potentials in Es-1 occurred during a sustained depolarization of A-P-1, suggesting that Es-1 received some inhibitory synaptic input from A-P-1. The amplitude of these slow synaptic hyperpolarizations was dependent on graded changes in the depolarization of A-P-1. However, presynaptic spikes in A-P-1 were not followed by discrete synaptic potentials in Es-1. These results suggest that graded photoresponses of A-P-1 could produce the slow hyperpolarization of Es-1 and that the photoresponse of A-P-1 is thereby transmitted to Es-1 with reversed polarity. The differing responses of Es-1 to light of different wavelengths may have a role in colour discrimination.

Introduction

Extra-ocular photoreception in molluscs was first demonstrated in photoresponsive neurones in *Aplysia fasciata* ganglia (Arvanitaki and Chalazonitis, 1961), and later in another opisthobranch mollusc, *Onchidium verruculatum* (Hisano *et al.* 1972*a*). A considerable amount of information has since been obtained about the conductance and phototransduction mechanisms involved in the generation of photoresponses (receptor potentials) by these extra-ocular photoreceptors in *Aplysia* (Brown and Brown, 1973; Brown *et al.* 1977; Andresen and Brown, 1979)

* To whom reprint requests should be addressed.

Key words: molluscan extra-ocular photoreceptors, colour discrimination, graded synaptic transmission, *Onchidium verruculatum*.

and *Onchidium* (Gotow, 1989; Nishi and Gotow, 1989; Gotow and Nishi, 1991). However, the functional importance of such photoresponsive neurones remains obscure (Strumwasser, 1973; Block *et al.* 1974). In *Onchidium*, it is suggested that the photoresponsive neurones play a role in regulating the transmission of tactile sensory information that releases the mantle-levating movement reflex (Gotow *et al.* 1973).

In the present study, we re-examined the photoresponsive neurone that has been named Es-1 by Hisano *et al.* (1972*a*). Our results show that Es-1 receives graded inhibitory synaptic inputs from another photoresponsive neurone, A-P-1, and is depolarized or hyperpolarized depending on whether the incident light is predominantly of a longer or shorter wavelength.

Materials and methods

Experimental animals, the opisthobranch mollusc *Onchidium verruculatum* Cuvier (10–15 g), were collected from the intertidal zone of Sakurajima in Kagoshima, Japan. The animals were kept in a natural seawater aquarium bubbled with air at 18–23 °C. They survived well for several months without feeding. The circumoesophageal ganglia were exposed by dissecting through the mid-dorsal surface of the mollusc and were isolated after the overlying connective tissue had been removed. This allowed the cell bodies in the ganglion to be visualized. To facilitate impalement of the cells by microelectrodes, the surface of each isolated ganglion complex was softened with 0.1 % Pronase (a nonspecific protease, 537088; Calbiochem) in saline for 5 min, and then rinsed in saline without Pronase for 30 min. The ganglia were then pinned dorsal side up on the wax base of a 1 ml chamber and perfused continuously with saline. In some experiments, the initial axonal segment of Es-1 was exposed by carefully removing the neighbouring neural or glial cells with forceps and fine tapered glass rods, and then its cell body and initial segment (about 300 μm in length) were cut free from the ganglion. These procedures were performed under dim red light or in total darkness.

The composition of the normal saline was (in mmol l^{-1}): NaCl, 450; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50. This saline was adjusted to pH 7.8 with 10 mmol l^{-1} THAM (Tris-hydroxymethyl-aminomethane) and HCl. To block chemical synapses, the ganglia were perfused with a Ca^{2+}-free, high-Mg^{2+} solution, which contained the following (in mmol l^{-1}): NaCl, 360; KCl, 10; $MgCl_2$, 120; adjusted to pH 7.8 with the same THAM and HCl. A chelating agent was not added to this Ca^{2+}-free saline. The temperature of the bathing solution ranged from 21 to 24 °C.

For intracellular recording, one or two single microelectrodes filled with 2.5 mol l^{-1} KCl, and with a resistance of less than 20 MΩ, were inserted under visual control into the same neurone. When two electrodes were used, current was applied to the cell through one electrode, while the second electrode was used for recording. In some experiments, a single electrode filled with a bridge circuit served for both current injection and recording. A 2.5 mol l^{-1} KCl–agar, Ag–AgCl bridge was placed in the chamber as an indifferent electrode. In some

experiments, the voltage-clamp technique described by Gotow (1989) was used. Voltage and current signals were recorded using a conventional differential d.c. amplifier and cathode-ray oscilloscope, stored on a digital tape recorder (DAT), and plotted on a penwriter recorder.

The general method of photostimulation has been described in detail elsewhere (Gotow, 1989). White or monochromatic light from a tungsten quartz–iodide lamp (6.3 V, 45 W) was used for stimulation. The monochromatic light was focused through a monochromator, but the white light was focused directly. Light was transmitted to the preparation *via* a fibre-optic bundle (0.5 cm in diameter). The light intensity was controlled by neutral-density filters, and the radiant energy flux was measured at the preparation with a radiometer (SJI, model 4090).

Micropipettes filled with 2.5 % Lucifer Yellow (CH type, Sigma) in distilled water were used for the intracellular staining of cells. The dye was injected by hyperpolarizing current pulses (1–10 nA, 0.5 s, 1 Hz) delivered for 5–8 h. The circumoesophageal ganglia were subsequently fixed in phosphate-buffered 4 % formaldehyde (pH 7.4) at 4 °C for at least 8 h, dehydrated through a standard alcohol series, and finally cleared in methyl salicylate. The stained cells were viewed in cleared whole mounts under a fluorescence microscope.

Results

Re-evaluation of the photoresponsive neurone Es-1

Hisano *et al.* (1972*a*) proposed that a photoexcitatory neurone Es-1 identified in the abdominal ganglion has a secondary role in light sensing because it receives excitatory inputs from primary photoexcitatory neurones. Here we investigated the possibility that Es-1 itself responds to light.

Exposure of the ganglion to Ca^{2+}-free, high-Mg^{2+} saline, which blocks chemical synapses and thus inhibits neural input, was found greatly to enhance the excitatory (depolarizing) response of Es-1 to illumination of the whole ganglion. In addition, the excitatory response to light stimulation was also repeatedly obtained in isolated Es-1 neurones (see Materials and methods). These results strongly suggest that the excitatory photoresponse of Es-1 is a primary (direct) response to light and is not caused by synaptic inputs. Our findings also suggest that Es-1 functions as an extra-ocular photoreceptor, as does the photoexcitatory neurone A-P-1, which is located in the same ganglion (Gotow, 1989).

Hisano *et al.* (1972*a*) have shown that, although Es-1 is usually excited by light, it is sometimes inhibited following repeated photic stimulation. In the present study, we describe inhibitory inputs onto Es-1 from A-P-1 that could underlie this inhibition. When Es-1 was isolated chemically or mechanically from the ganglion, it always showed an excitatory depolarizing photoresponse.

When Es-1 cells were impaled with microelectrodes and dark-adapted for 30–60 min, the resting membrane potential was −40 to 50 mV. The input resistance at this resting potential ranged from 6 to 8 MΩ, as calculated from the voltage responses to 10–30 mV hyperpolarizing current pulses of 1 s duration. The

time constant calculated from the time course of these voltage responses ranged from 190 to 210 ms. Calculations using these membrane resistance and time constant values yielded a membrane capacitance for Es-1 of 0.027–0.029 μF.

Comparison of the photoresponses of Es-1 and A-P-1

Gotow (1989) reported that the extra-ocular photoreceptor A-P-1 responded to light with an excitatory (depolarizing) receptor potential resulting from a decrease in membrane conductance to K^+. This conductance decrease associated with a photoresponse was the first to be demonstrated in any invertebrate ocular or extra-ocular photoreceptor. Accordingly, the photoresponse of Es-1 (a similar primary photoreceptor) was compared with that of A-P-1.

Some properties of the response of Es-1 caused by white light stimulation are shown in Fig. 1. All records were obtained in Ca^{2+}-free, high-Mg^{2+} solution in order to suppress inputs from presynaptic photoresponsive cells. A 30-s illumination induced a slowly developing depolarization (excitation) after a delay of about 500 ms. This reached a peak in about 15 s and then returned to the initial (dark-adapted) level more slowly (Fig. 1Ai). The amplitude of the depolarization (generator potential) was dependent on the light intensity, and it led to action potentials under appropriate conditions. The depolarizing photoresponse was associated with a decrease in membrane conductance as indicated by a 15–20 % increase in the amplitude of electrotonic potentials generated by 1-s constant-current pulses (Fig. 1Aii). Direct depolarization (in the dark) to nearly the peak potential obtained during the photoresponse produced little change in membrane conductance (Fig. 1Aiii). This showed that the decrease in conductance associated with the photoresponse was independent of the light-induced changes in membrane potential; i.e. that it was not due to membrane rectification in Es-1. When Es-1 was voltage-clamped at the dark-adapted resting potential, illumination was followed by a slow inward current which had a time course similar to that of the voltage photoresponse in an unclamped Es-1 (Fig. 1Aiv).

Fig. 1B shows the photoresponse to white light stimulation (1 min duration) at different membrane potentials achieved by passing current across the Es-1 membrane. In these experiments, when identical stimuli were presented at appropriate intervals to a cell held in the dark at a fixed level of resting membrane potential, a constant, reproducible light-induced depolarization was obtained. As shown in Fig. 1Bi–iv, the amplitude of the light-induced depolarization (photoresponse) gradually decreased as the membrane potential was hyperpolarized from -45 to -70 mV and became zero at approximately -70 mV, which is close to the K^+ equilibrium potential of A-P-1 cells in normal saline (Gotow, 1989). However, the polarity of the photoresponse was seldom reversed even if the cell was hyperpolarized further, showing that the Es-1 photoresponse is voltage-dependent and is similar to the nonlinear voltage–current relationship of the steady-state A-P-1 photoresponse (Gotow, 1989). Similar results were obtained when five other Es-1 cells were tested. These results suggest that the ionic mechanism

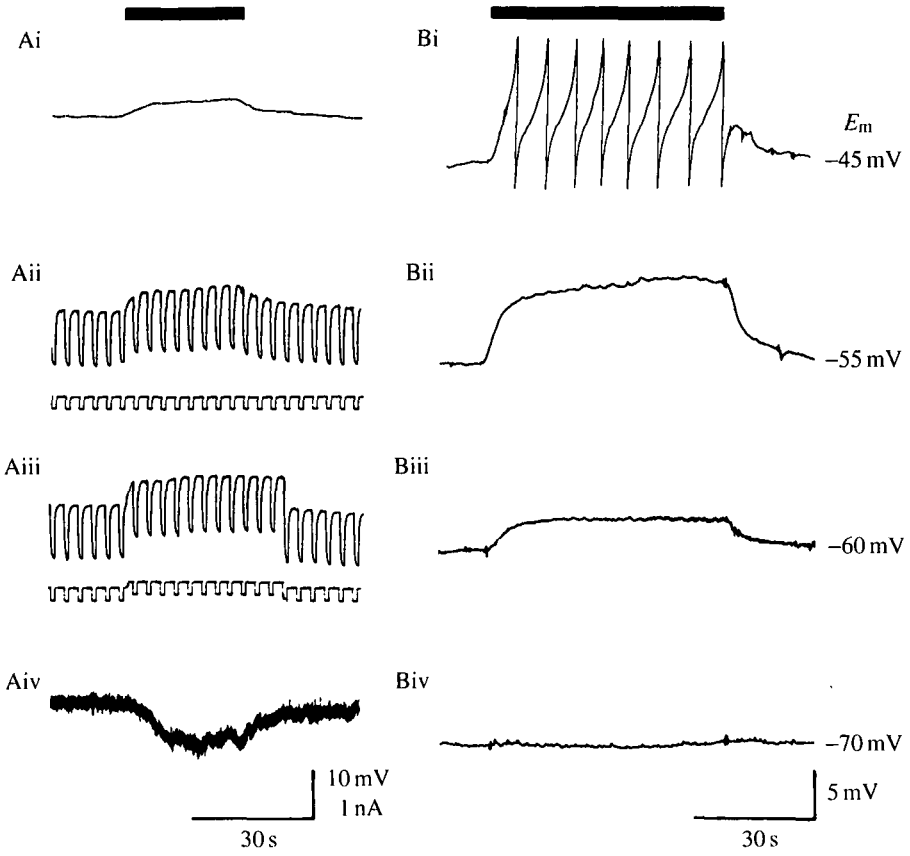

Fig. 1. Responses of Es-1 to white light. The preparation was perfused with low-Ca^{2+}, high-Mg^{2+} saline to block any possible presynaptic contribution. The light stimulus is indicated by horizontal bars (30 s in A, 1 min in B) at the top. The stimulus intensity was 1.5×10^{-3} W cm^{-2} in A and 3×10^{-3} W cm^{-2} in B. (Ai) Depolarizing response at the resting potential (−43 mV). (Aii) Potential changes produced by short constant-current pulses (1 nA, 1 s duration) are shown before, during and after the photoresponse. (Aiii) Potential changes caused by the same pulses during passive depolarization to nearly the same potential as that reached during the photoresponse. (Aiv) Inward current response of a cell voltage-clamped at the membrane potential of −45 mV. (B) Effect of membrane potential on the photoresponse. The resting membrane potential (E_m) was held at −45 mV in Bi, −55 mV in Bii, −60 mV in Biii and −70 mV in Biv. All records are from a single neurone. The tops of the action potentials have been deleted in Bi and all subsequent penwriter records.

underlying the Es-1 photoresponse is suppression by light of a voltage- (and time-) dependent K^+ current (conductance).

The response of Es-1 to various wavelengths of monochromatic light in the range 400–700 nm was similar to the response to white light, and the time courses of subthreshold responses to any two wavelengths presented individually were identical when the light intensity was suitably adjusted. The spectral sensitivity of

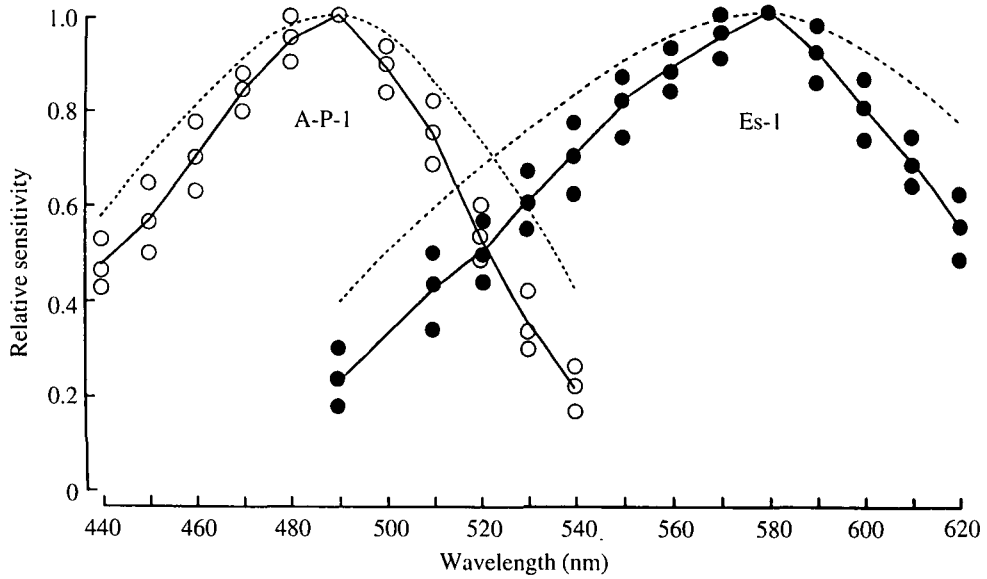

Fig. 2. Spectral sensitivity curves of two extra-ocular photoreceptors, Es-1 (filled circles) and A-P-1 (open circles). The data for A-P-1 are taken from Gotow (1989) and are reproduced for comparison. The measurements for Es-1 were made in low-Ca^{2+}, high-Mg^{2+} saline to avoid the contribution of presynaptic activity. The ordinate is the relative quantum sensitivity and the abscissa shows the wavelength. Each set of points at each wavelength was normalized to a value of unity at 580 nm for Es-1 (and 490 nm for A-P-1). The solid curves are the mean relative sensitivities determined from three cells. The broken lines are Dartnall nomograms for visual pigments with peak absorbances at 490 nm and 580 nm.

Es-1 was determined by measuring the intensity of a 10-s light exposure necessary to evoke a response of constant amplitude (3–5 mV) at each wavelength. Sensitivity was calculated as the reciprocal of this intensity in quanta $cm^{-2}s^{-1}$ corresponding to the constant response amplitude at each wavelength. Fig. 2 shows the relative spectral sensitivity of three different Es-1 cells normalized against their sensitivity at 580 nm. All measurements were made in Ca^{2+}-free, high-Mg^{2+} solution to avoid the effect of the presynaptic neurones. Data for A-P-1 (from Gotow, 1989) are reproduced for comparison.

Es-1 was maximally sensitive at about 580 nm. This value was similar to the result obtained by assessing current responses under voltage-clamped conditions. The dashed curve is the nomogram (Dartnall, 1953) for a hypothetical visual pigment with an absorption maximum at 580 nm; it deviates somewhat from the measured action spectrum curve. Comparison with A-P-1 (from Gotow, 1989) suggests that the depolarizing photoresponses of Es-1 and A-P-1 are mediated by different photopigments.

Modulation of the direct photoresponse of Es-1 by A-P-1

As described above, the direct photoresponse of Es-1 was a slow depolarizing,

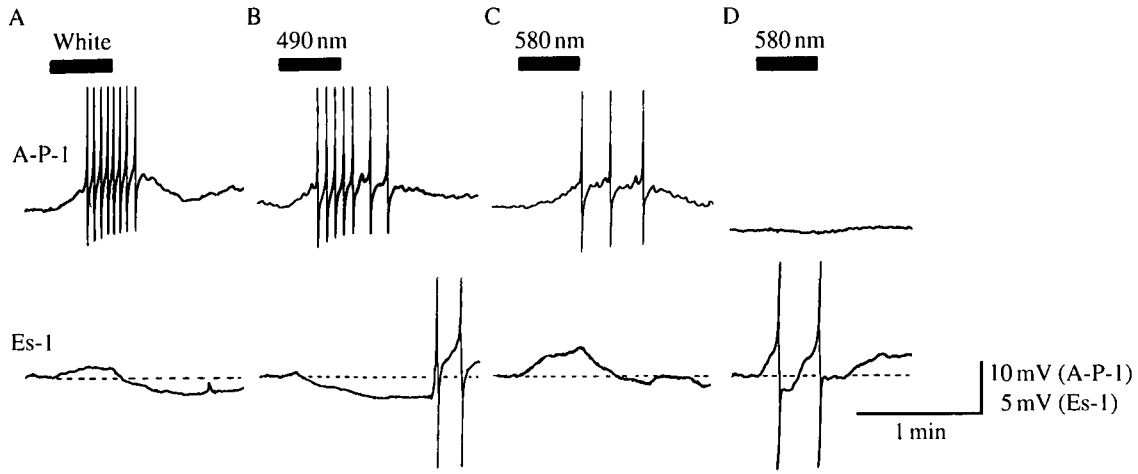

Fig. 3. Photoresponses of A-P-1 and Es-1 recorded simultaneously. Each light stimulus (indicated by bars at the top) was 30 s in duration. The intensities were 3×10^{-3} W cm^{-2} in A (white light), 1.2×10^{-4} W cm^{-2} in B (490 nm) and 1×10^{-4} W cm^{-2} in C and D (580 nm). Thus, in terms of the number of photons, the intensities of the 490 and 580 nm light were almost the same (0.3×10^{15} quanta cm^{-2} s^{-1}). The initial resting potential of A-P-1 was 50 mV in A, B and C and −60 mV in D, and that of Es-1 was −45 mV.

monotonic wave, which was graded by the light intensity at any wavelength until spikes were superimposed on the depolarization (for brighter stimuli). However, when perfused with normal saline to preserve normal synaptic activity, Es-1 showed a more complicated photoresponse. An example is shown in Fig. 3, in which A-P-1 is recorded simultaneously with Es-1.

Stimulation by white light of Es-1 evoked only a small depolarization (Fig. 3A). In contrast, the response of Es-1 in Ca^{2+}-free, high-Mg^{2+} saline to the same light stimulus was a large depolarization associated with spikes (Fig. 1Bi). The response of Es-1 to blue-green light (490 nm) was a hyperpolarization opposite in polarity to the direct photoresponse of Es-1 in Ca^{2+}-free, high-Mg^{2+} saline (Fig. 3B). In yellow light (580 nm) Es-1 was depolarized and spiked when the photoresponse of A-P-1 was suppressed by an extrinsic hyperpolarizing current (Fig. 3D), but Es-1 did not spike when input from A-P-1 was present (Fig. 3C). These results imply that the photoresponse of Es-1 is affected by input from A-P-1 and by the colour of the stimulating light and not just by its intensity (since intensity was maintained at a similar value for stimuli at both 490 and 580 nm). In comparison, the A-P-1 cell recorded simultaneously with Es-1 (Fig. 3A–C) showed only simple depolarizations with superimposed spikes and, as reported previously (Gotow, 1986, 1989), there was no significant difference in its photoresponse when it was bathed in normal saline or Ca^{2+}-free, high-Mg^{2+} saline.

The observation (Fig. 3D) that hyperpolarization (inhibition) of A-P-1 increases the photoresponse of Es-1 suggests that A-P-1 may inhibit Es-1. Such an

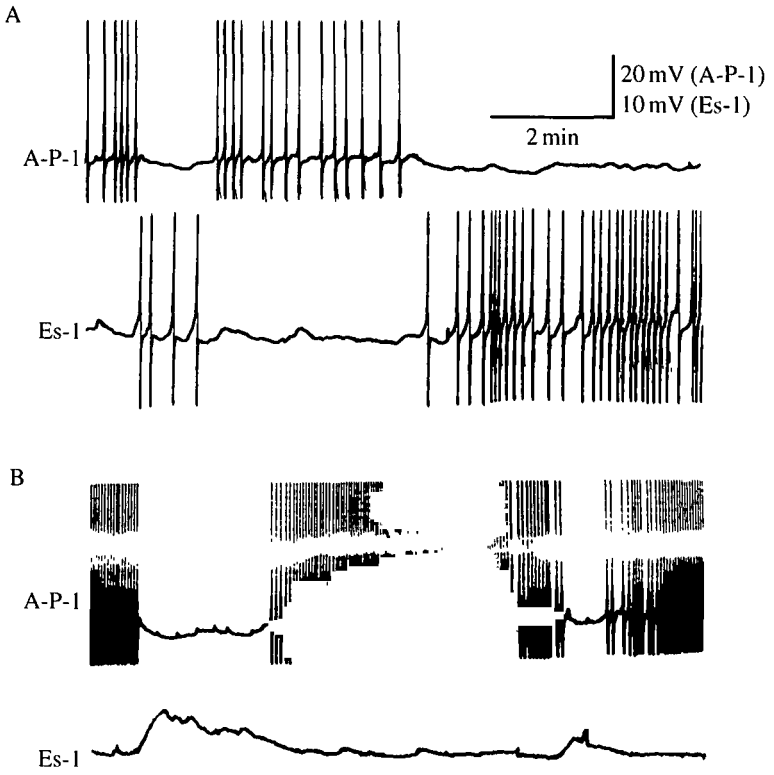

Fig. 4. Spontaneous activity of A-P-1 and Es-1 recorded simultaneously. A and B are from different preparations. The initial resting potential of A-P-1 was −43 mV in A and −40 mV in B; that of Es-1 was −40 mV in A and −50 mV in B. For an explanation, see the text.

inhibitory input from A-P-1 could explain the colour-dependence of the Es-1 photoresponse.

Fig. 4 shows simultaneous recordings from two other pairs of A-P-1 and Es-1 cells that show spontaneous changes of membrane potential. As seen in Fig. 4A, the spontaneous discharge of Es-1 is interrupted when A-P-1 fires, although each spike of A-P-1 was not followed by a unitary inhibitory potential in Es-1. Furthermore, a spontaneous depolarization of Es-1 appeared during the sustained interval in the spontaneous discharge of A-P-1 (Fig. 4B).

These results also suggest that the photoresponse of Es-1 is influenced by inhibitory input from A-P-1.

Synaptic inhibition of Es-1 by A-P-1

To confirm more directly the above inhibitory interactions between A-P-1 and Es-1, both neurones were impaled simultaneously and stimulated intracellularly by the injection of a given current pulse (Fig. 5).

A sustained subthreshold depolarization of A-P-1, induced by a long current

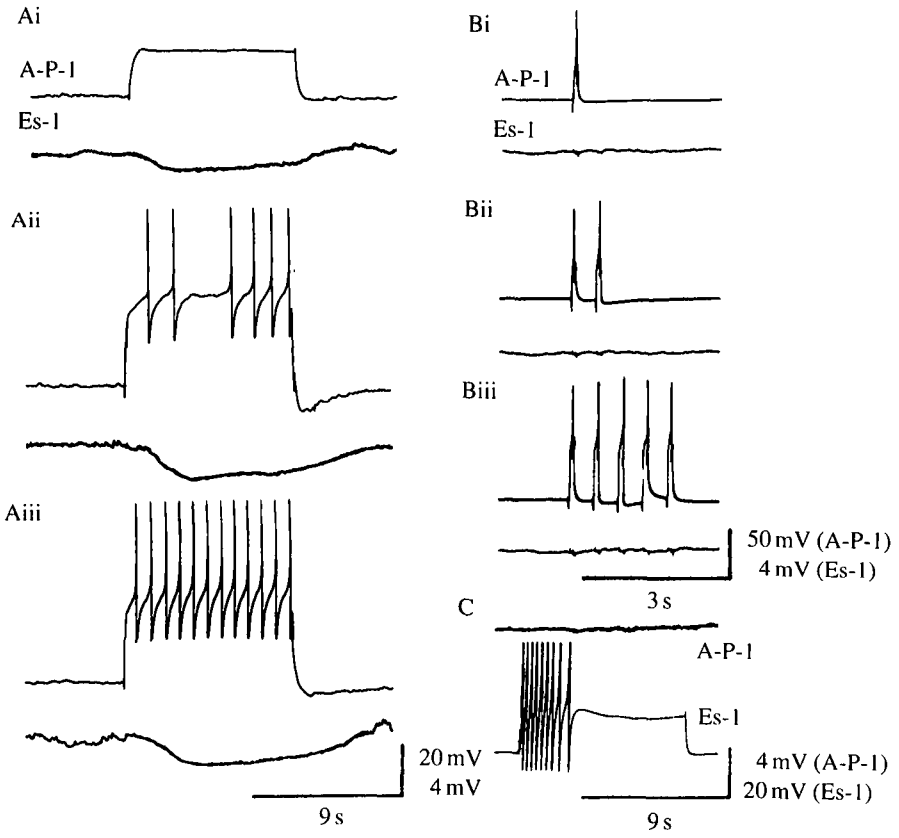

Fig. 5. Graded synaptic transmissions from A-P-1, which produced hyperpolarization in Es-1. A-P-1 and Es-1 were recorded simultaneously. (A) Slow inhibitory synaptic potentials in Es-1 produced by long (10 s) subthreshold (Ai) and suprathreshold (Aii, Aiii) depolarizations in A-P-1. The depolarizing current intensity applied to A-P-1 was 3 nA in Ai, 6 nA in Aii and 7 nA in Aiii. (B) A-P-1 spikes evoked by short current pulses of 100 ms and 10 nA had no effect on Es-1. (C) Depolarization of Es-1 (10 s, 5 nA) had no effect on A-P-1. In A and B, the initial membrane potential of A-P-1 was −50 mV, but before presynaptic stimulation the potential was altered to −80 mV to suppress tonic transmitter release due to graded transmission. The resting potential of Es-1 was held at −46 mV throughout. All records are from the same pair of A-P-1 and Es-1 cells.

pulse (10 s), evoked a slowly developing hyperpolarization of Es-1 (Fig. 5Ai). The amplitude of this slow hyperpolarization increased gradually as stronger (3–7 nA) current pulses were applied to A-P-1 (Fig. 5Aii,iii). Conversely, no depolarization or hyperpolarization of A-P-1 was observed when Es-1 was depolarized sufficiently to evoke spikes (Fig. 5C). These findings suggest that Es-1 is postsynaptic to A-P-1 and receives some inhibitory synaptic inputs from it, but that there is no feedback from Es-1 to A-P-1.

Individual spikes in A-P-1 did not cause discrete synaptic potentials in Es-1 (Fig. 5Aii,iii). Furthermore, although the frequency of spikes in A-P-1 increased

according to the amount of depolarizing current applied to A-P-1, the sustained depolarization (generator potential) of A-P-1 was almost the same for suprathreshold currents of two different intensities. The frequency of spikes in A-P-1 had no detectable effect on the amplitude or duration of the slow hyperpolarization of Es-1 (compare Fig. 5Aii with Aiii).

These results would be expected if the presynaptic spikes were blocked during their conduction to the presynaptic terminals (e.g. because of the low-pass filtering properties of the spike-blocking regions). If this were the case, then spike activity would not be required for synaptic transmission from A-P-1 to Es-1. Indeed, spikes in A-P-1 evoked by short (100 ms) depolarizing current pulses (which were shorter than the membrane time constant of about 150 ms) had no detectable effect on the membrane potential of Es-1 (Fig. 5Bi). Furthermore, no summation of synaptic potentials in Es-1 was observed following the repetitive firing of A-P-1 (Fig. 5Bii,Biii).

The slow synaptic transmission between A-P-1 and Es-1 is likely to be mediated by chemical, rather than electrical, synapses for the following reasons. (1) Application of a long depolarizing current to the postsynaptic Es-1 produced no change in the membrane potential of the presynaptic A-P-1 (Fig. 5C). This would be unlikely if the synapse were electrical. (2) The slow inhibitory synaptic potential in Es-1 was decreased by hyperpolarization of Es-1 itself and reversed by further hyperpolarization (data not shown). This finding could be expected to result from a change in membrane conductance, as is the case in other chemical synapses. (3) Replacing the normal saline with Ca^{2+}-free, high-Mg^{2+} saline blocked the inhibition of Es-1, as would be expected for a chemical synapse.

Staining of Es-1 and A-P-1 with Lucifer Yellow

The axonal branches arising from A-P-1 and Es-1 have previously been visualized by injecting cobalt (Gotow, 1975, 1989). In the present study, A-P-1 and Es-1 were simultaneously stained by the intracellular injection of a fluorescent dye, Lucifer Yellow, in order to clarify the relative spatial arrangements of their cell bodies and axonal branches in the same preparation. As shown in Fig. 6, the main axonal branching pattern of each extra-ocular photoreceptor was basically the same as that shown previously by cobalt staining. In addition, A-P-1 had some branches that remained within the ganglion, so that the axonal branches of A-P-1 did not run towards the nerves leaving the ganglion. Lucifer Yellow staining also showed a strikingly complex array of fine branches arising from the main axons of A-P-1 and Es-1 in the neuropiles of the abdominal ganglion and the left and right pleuro-parietal ganglia, which was never revealed by cobalt staining. This complex network of fine branches frequently contained swellings resembling varicosities. Many fine terminal branches from A-P-1 seemed to be in contact with fine branches of Es-1 in all three ganglia. However, movement of Lucifer Yellow from A-P-1 to Es-1 cells or *vice versa*, as would be predicted from the above electrophysiological data, was not observed after separate dye injection into A-P-1 or Es-1.

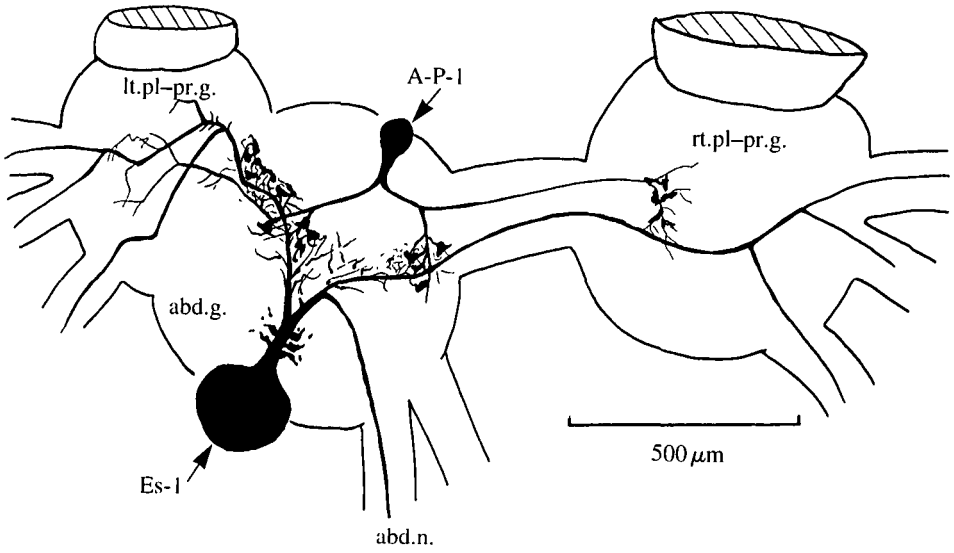

Fig. 6. Sketch (to scale) of the antero-dorsal surface of the circumoesophageal ganglia showing A-P-1 and Es-1 cells stained simultaneously with Lucifer Yellow. The stained cells were reconstructed from several different focal planes viewed in whole mounts under a fluorescence microscope. The main axonal branches arising from the cell bodies of A-P-1 and Es-1 were nearly the same as those previously detected with cobalt staining (Gotow, 1975, 1989). Note also a striking complex network of fine branches containing some varicosities, which was not revealed by cobalt staining. The left and right pleuro-parietal ganglia and the abdominal ganglion are abbreviated as lt.pl–pr.g., rt.pl–pr.g. and abd.g., respectively. The abdominal nerve is labelled abd.n.

Discussion

The photoresponse of the Es-1 neurone in the abdominal ganglion of *Onchidium verruculatum* was assumed to be a secondary effect caused by a synaptic potential arising from a primary photoresponsive neurone (Hisano *et al.* 1972*a*). However, this study presents evidence that Es-1 functions as an extra-ocular photoreceptor (a primary photoresponsive neurone), as does another extra-ocular photoreceptor, A-P-1, found in the same ganglion (Gotow, 1986). In Es-1 cells, an excitatory (depolarizing) receptor potential (the photoresponse) is associated with a decrease in membrane conductance; this is a common characteristic of the photoresponses of vertebrate photoreceptors (and of A-P-1), but not of other invertebrate photoreceptors. The depolarizing photoresponse of Es-1 was replaced by an inward current when the cell was voltage-clamped at the resting potential level. This current decreased with hyperpolarizing shifts of the membrane potential, until no response was observed at about −70 mV, but its polarity did not undergo reversal even with further hyperpolarization. This voltage dependence of the Es-1 photoresponse is similar to that of the light-suppressible, voltage- (and time-) dependent K^+ current previously reported for the A-P-1 photoresponse (Gotow, 1989). From these and previous results, we conclude that

the photoresponse of Es-1 may also be due to the suppression of a voltage-dependent K^+ current.

The direct (primary) photoresponse of Es-1 obeyed the principle of univariance (Naka and Rushton, 1966) and had a peak sensitivity at 580 nm, which differed from the action spectrum peak of A-P-1 (490 nm). Light at 579 nm has been found most effectively to depolarize the photoresponsive A cell of another opisthobranch mollusc, *Aplysia fasciata* (Arvanitaki and Chalazonitis, 1961), but this cell is also hyperpolarized by light at 490 nm, showing non-univariance with respect to wavelength.

The general conclusion from the present study is that the primary extra-ocular photoreceptor Es-1 receives an inhibitory input from another primary photoreceptor, A-P-1, which is presynaptic to it, but that Es-1 does not have any feedback effect on A-P-1. This inhibitory input apparently causes Es-1 to produce photoresponses of opposite polarity in response to stimulation by light of different colours. A tentative mechanism for this colour-dependence of the Es-1 response is presented in Fig. 7.

The slow inhibitory (hyperpolarizing) potentials acting on Es-1 were produced

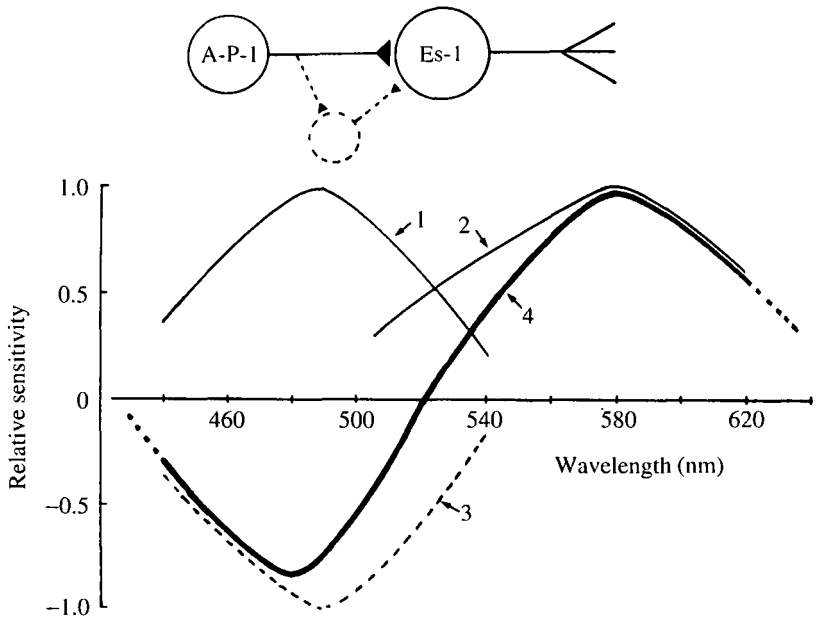

Fig. 7. A tentative mechanism to explain the modification of Es-1 photoresponses by A-P-1. Curves 1 and 2 reproduce the spectral sensitivity curves of A-P-1 and Es-1, respectively. The dashed curve (3) is an inversion of curve 1 and shows the spectral range producing the synaptic hyperpolarization of Es-1. Curve 4 shows the colour-dependence of the photoresponse of Es-1 and was obtained by combining curves 2 and 3. Upper inset: a diagram of the proposed inhibitory synaptic connections between A-P-1 and Es-1. As indicated by the broken lines, it is not clear whether these connections are monosynaptic. For details, see the text.

by long (10 s), rather than short (100 ms), depolarizing pulses applied to A-P-1, so that the presynaptic spikes in A-P-1 evoked by short pulses had no effect on Es-1. The resulting slow synaptic hyperpolarization of Es-1 was activated at membrane potentials more negative than those of the spikes in the presynaptic A-P-1 and its amplitude apparently depended on graded changes during sustained depolarization of A-P-1. However, the more detailed relationships between presynaptic and postsynaptic voltages have not yet been investigated. While this contrasts with several other well-known synaptic potentials mediated by presynaptic spikes, a similar type of synaptic transmission without presynaptic spikes has been reported in the dragonfly median ocellus (Chappell and Dowling, 1972).

The data show that the graded depolarizing photoresponse of A-P-1 causes a graded hyperpolarization of Es-1 (i.e. the photoresponse of A-P-1 is transmitted in an inverted form to Es-1). Thus, curve 3 in Fig. 7, obtained by reversing the spectral sensitivity of the A-P-1 photoresponse, should show the spectral range that causes the hyperpolarization of Es-1. In the absence of any synaptic stimulation from A-P-1 and/or other cells, Es-1 itself responds to light with a pure depolarization that develops gradually and has a peak sensitivity at 580 nm. Thus, under normal conditions, Es-1 is hyperpolarized by blue-green light (490 nm) (which is most effectively absorbed by A-P-1 but poorly detected by Es-1) and is depolarized by yellow light (580 nm), to which it is maximally sensitive but which has a negligible effect on A-P-1. The entire spectral characteristics of the colour-dependent photoresponses of Es-1 can be predicted from curve 4 in Fig. 7, which was obtained by combining these effects. Thus, Es-1 apparently functions to discriminate between shorter and longer wavelengths of light rather than its brightness. Similar colour-opponent (dependent) cells that are hyperpolarized by some wavelengths and depolarized by others occur in the primary retinal photoreceptors of the honeybee (Menzel and Blakers, 1976) and turtle (Fuortes *et al.* 1973), although the mechanisms underlying their responses are not understood.

The present study did not clarify whether the connection between A-P-1 and Es-1 was monosynaptic, although the data suggest that the hyperpolarization of Es-1 is mediated by chemical synaptic transmission. However, the monosynaptic or polysynaptic nature of the pathway does not influence our explanation of the colour-dependent photoresponse of Es-1.

The colour-dependence of the Es-1 response may play a role in regulating neural or behavioural activity patterns of *Onchidium verruculatum*. Our previous work (Gotow *et al.* 1973) has shown that Es-1 is one of the motor neurones acting in the mantle-levating reflex, which is released by tactile stimulation of the mantle. Thus, the primary photoresponsive neurone Es-1 is not only a second-order neurone receiving tactile or other sensory synaptic inputs, but it is also the motor neurone innervating the mantle and mesopodium. Because the present study indicated that Es-1 was depolarized or hyperpolarized by light, depending on its colour, it is possible that the mantle-levating reflex is modulated by the colour-opponent photoresponses of this neurone.

Es-1 may also be involved in other locomotive behaviour patterns (Gotow, 1975). For instance, *Onchidium* is a diurnally active mollusc that lives in the littoral zone and at low tide crawls over the exposed shoreline to obtain food or reproduce. It is usually more active under light conditions when the longer wavelengths predominate (such as during the early morning, towards evening or on cloudy days) than when shorter wavelengths dominate. Accordingly, certain behaviour patterns of *Onchidium* may be modulated by differences in the wavelength of natural incident light. The extra-ocular photoreceptors A-P-1 and Es-1 are possible candidates for regulating such behavioural changes.

In addition to the extra-ocular photoreceptor neurones referred to here, *Onchidium verruculatum* has a multiple photoreceptor system located in different regions of the body, including the stalk eyes, dorsal eyes and dermal eyes (Katagiri *et al.* 1985). These eyes mediate reactions, such as cessation of locomotion, withdrawal of the stalk eyes and contraction of the mantle, in response to a sudden increase or decrease of diffuse illumination (Hisano *et al.* 1972*b*). Thus, they seem to function exclusively in the general perception of luminosity, but not in colour discrimination.

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and from the Scientific Promotive Foundation for Southwest Pacific Area, Kagoshima University. This work was also submitted as a part of the Doctoral dissertation of T.N. at Hiroshima University.

References

ANDRESEN, M. C. AND BROWN, A. M. (1979). Photoresponses of a sensitive extraretinal photoreceptor in *Aplysia*. *J. Physiol., Lond.* **287**, 267–282.

ARVANITAKI, A. AND CHALAZONITIS, N. (1961). Excitatory and inhibitory processes initiated by light and infra-red radiations in single identifiable nerve cells (giant ganglion cells of *Aplysia*). In *Nervous Inhibition* (ed. E. Florey), pp. 194–231. Oxford: Pergamon Press.

BLOCK, G. D., HUDSON, D. J. AND LICKEY, M. E. (1974). Extraocular photoreceptors can entrain the circadian oscillator in the eye of *Aplysia*. *J. comp. Physiol.* **89**, 237–249.

BROWN, A. M., BRODWICK, M. S. AND EATON, D. C. (1977). Intracellular calcium and extra-retinal photoreception in *Aplysia* giant neurons. *J. Neurobiol.* **8**, 1–18.

BROWN, A. M. AND BROWN, H. M. (1973). Light response of a giant *Aplysia* neuron. *J. gen. Physiol.* **62**, 239–254.

CHAPPELL, R. L. AND DOWLING, J. E. (1972). Neural organization of the median ocellus of the dragonfly. I. Intracellular electrical activity. *J. gen. Physiol.* **60**, 121–147.

DARTNALL, H. J. A. (1953). The interpretation of spectral sensitivity curves. *Bri. med. Bull.* **9**, 24–30.

FUORTES, M. G. F., SCHWARTZ, E. A. AND SIMON, E. J. (1973). Colour-dependence of cone responses in the turtle retina. *J. Physiol., Lond.* **234**, 199–216.

GOTOW, T. (1975). Morphology and function of the photoexcitable neurones in the central ganglia of *Onchidium verruculatum*. *J. comp. Physiol.* **99**, 139–152.

GOTOW, T. (1986). Decrease of K^+ conductance underlying a depolarizing photoresponse of a molluscan extraocular photoreceptor. *Experientia* **42**, 52–54.

GOTOW, T. (1989). Photoresponses of an extraocular photoreceptor associated with a decrease in membrane conductance in an opisthobranch mollusc. *Brain Res.* **479**, 120–129.

GOTOW, T. AND NISHI, T. (1991). Roles of cyclic GMP and inositol trisphosphate in phototransduction of the molluscan extraocular photoreceptor. *Brain Res.* **557**, 121–128.

GOTOW, T., TATEDA, H. AND KUWABARA, M. (1973). The function of photoexcitive neurones in the central ganglia for behavioral activity of the marine mollusc, *Onchidium verruculatum*. *J. comp. Physiol.* **83**, 361–376.

HISANO, N., TATEDA, H. AND KUWABARA, M. (1972*a*). Photosensitive neurones in the marine pulmonate mollusc *Onchidium verruculatum*. *J. exp. Biol.* **57**, 651–660.

HISANO, N., TATEDA, H. AND KUWABARA, M. (1972*b*). An electrophysiological study of the photo-excitative neurones of *Onchidium verruculatum in situ*. *J. exp. Biol.* **57**, 661–670.

KATAGIRI, Y., KATAGIRI, N. AND FUJIMOTO, K. (1985). Morphological and electrophysiological studies of a multiple photoreceptive system in a marine gastropod, *Onchidium*. *Neurosci. Res. Suppl.* **2**, S1–S15.

MENZEL, R. AND BLAKERS, M. (1976). Colour receptors in the bee eye – morphology and spectral sensitivity. *J. comp. Physiol.* **108**, 11–33.

NAKA, K.-I. AND RUSHTON, W. A. H. (1966). S-potentials from colour units in the retina of fish (*Cyprinidae*). *J. Physiol., Lond.* **185**, 536–555.

NISHI, T. AND GOTOW, T. (1989). A light-induced decrease of cyclic GMP is involved in the photoresponse of molluscan extraocular photoreceptors. *Brain Res.* **485**, 185–188.

STRUMWASSER, F. (1973). Neural and humoral factors in the temporal organization of behavior. *Physiologist* **16**, 9–41.