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PHOTOSENSITIVE NEURONS IN MOLLUSKS

GORDANA KARTELIJA[1], M. NEDELJKOVIĆ[1] and LIDIJA RADENOVIĆ[2]

[1]*Siniša Stanković Institute for Biological Research,* 11000 Belgrade, Serbia and Montenegro; [2] *Department of Physiology and Biochemistry, Faculty of Biology, University of Belgrade*, 11000 Belgrade, Serbia and Montenegro

Abstract - In addition to regular photoreceptors, some invertebrates possess simple extraocular photoreceptors. For example, the central ganglia of mollusks contain photosensitive neurons. These neurons are located on the dorsal surface of the ganglia and based on their electrophysiological properties, it has been postulated that they are internal photoreceptors. Besides the eye, transduction of light also occurs in these extra-ocular photoreceptors. In the present work, we analyze the reactivity of these nerve cells to light and describe the underlying mechanism mediating the light-induced response.

Key words: *Aplysia*, 8-Br-cGMP, Ca^{2+} channel, cGMP, *Helix pomatia*, IBMX, identified neurons, phosphodiesterase inhibitor, photosensitive neurons, second messenger

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INTRODUCTION

Extraretinal photoreception is a widespread biological phenomenon occurring in a variety of excitable tissues such as neurons (Arvanitaki and Chalazonitis, 1961), axons (Kennedy, 1958), and cells located in the pineal and parietal organs (Tosini *et al.* 2000) and it is implicated in light-linked behavioral and hormonal responses (Menaker, 1972).

It is well known that some invertebrates possess simple photoreceptors, such as those in the caudal ganglion of the crayfish (Prosser, 1934), in the central ganglia of *Aplysia* (Arvanitaki and Chalazonitis, 1961), in the epistelar body of the octopus (Mauro and Bauman, 68), and in the parolfactory vesicles of the squid (Mauro and S-Knudsen, 1972). The central ganglia of the marine pulmonate mollusk, *Onchidium verruculatum* also contain photoexcitable neurons (Hisano *et al.* 1972). In *Onchidium*, some neurons, named photoexcitative neurons by Hisano *et al.* (1972), were excited by a light stimulus in an isolated ganglion preparation, while others – photoinhibitive neurons – were inhibited by it. We described earlier a class of photosensitive neurons in *Helix pomatia* subesophageal ganglia that respond to the onset of light with membrane depolarization (Pašić *et al.* 1977).

The photoexcitable neurons on the dorsal surface of the *Helix* subesophageal ganglia are assumed to be internal photoreceptors based on their electrophysiological properties. They are suitable for neurophysiological analyses because of their apparent simplicity. However, with the exception of the crayfish internal photosensitive neurons (Wilkens, 1988), little is known about their physiological role. Experiments studying photosensitive neurons can not only provide an understanding of the behavioural significance of photoexcitative neurons, but also be used for comparative study of the physiological mechanisms involved in the phototransduction of simple photosensory systems.

Photosensitive neurons of gastropods

Identification of photosensitive neurons

As mentioned above, Arvanitaki and Chalazonitis (1958) first identified photosensitive neurons in the ganglia of *Aplysia* and *Helix*. They have shown that neurons of these species react to a light stimulus with a change in the spontaneous action potential generation. Most previous investigations studying the effects

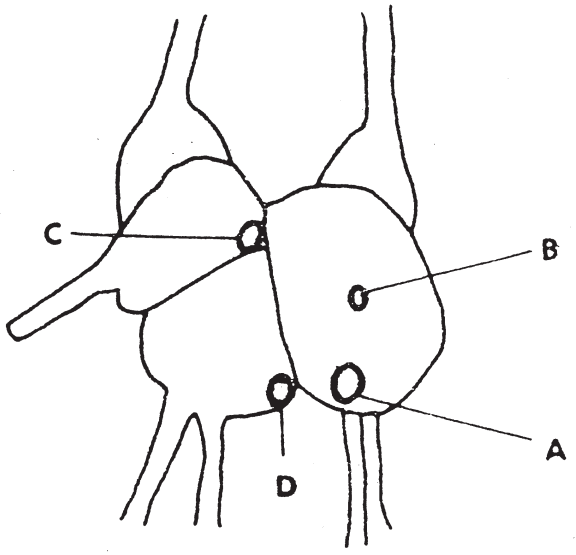

Fig. 1. Schematic presentation of subesophageal ganglia of *Helix pomatia* together with the position of identified neurons (A, B, C, and D) in which the effects of light were investigated. LP - left parietal ganglia, RP - right parietal ganglia, V - visceral ganglia.

of neuron photostimulation were carried out on the *Aplysia* abdominal ganglion, specifically on the R2 neuron, where light induces a slow membrane hyperpolarization (Zečević and Pačić, 1972; Brown and Brown, 1973; Brown *et al.* 1975). Reactivity to light was used as one of the identification criteria for some *Aplysia* abdominal ganglion nerve cells in the work of Frazier *et al.* (1967). Nelson *et al.* (1976) characterized the photoresponse of the R15 *Aplysia* neurosecretory neuron, and measured its spectral sensitivity by electrophysiological methods. Illumination of the R15 neuron with white light produced a slow membrane hyperpolarization, which apparently caused temporary inhibition of bursting activity. The hyperpolarization was found to be a function of both light intensity and wavelength. Photosensitive neurons were also described in the central ganglia of the marine pulmonate mollusk, *Onchidium verruculatum* (Hisano *et al.* 1972; Gotow *et al.* 1973).

According to our previous findings, the majority of *Helix pomatia* neurons located in subesophageal ganglia respond to light with a depolarization and an increase in action potential frequency (Pašić, 1975). The effect of photostimulation was investigated in four classes of identified neurons in *Helix pomatia* subesophageal ganglia (Fig. 1). The effects of photostimulation on the four tested cell types are presented in Fig. 2. Our recordings show that in three out of four tested neurons (A, C, and D), the

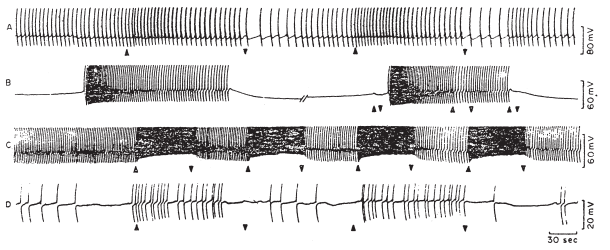

Fig. 2. Effect of photostimulation on neurons A, B, C, and D from Fig. 1. Light on and off at arrows. Note the discontinuity of record B, where a 300 sec period of recording was cut out between the brackets.

onset of light induced a depolarization and an in crement of action potential frequency that persisted during the entire period of illumination. During a subsequent dark period of the same duration, a hyperpolarization occurred and the action potential frequency decreased. Only in one of the tested neurons (B in Fig. 1) did illumination induce a hyperpolarization and a decrement of action potential frequency.

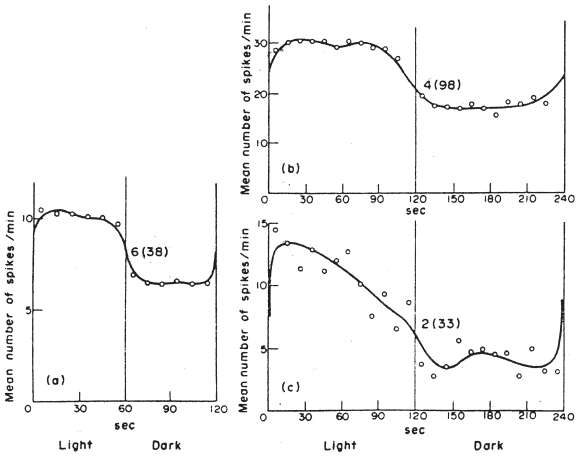

Fig. 3. Averaged reaction of three neurons to intermittent photostimulation. Curve a was obtained by averaging mean frequencies in 38 1-min light periods in experiments on neuron C in the left parietal ganglion. Curve b refers to four experiments on neuron A (Br neuron) in the right parietal ganglion. Curve c was obtained on two neurons (D) in the visceral ganglion. Each point on the curves represents the mean number of spikes/min (y) calculated by means of the formula $y = 60\ n/T$ at the chosen moment from the start of illumination or from its ending. T (being equal to 30 sec) is the time interval placed symmetrically around the chosen moment; n is the number of spikes in this time interval.

In experiments where several light/dark periods of equal duration were applied to the tested photosensitive neurons, the dynamics of action potential frequency increment during illumination and its decrement during darkness could be analyzed. The average action potential frequency of the three tested neurons during 1-min and 2-min illumination and during subsequent dark periods of

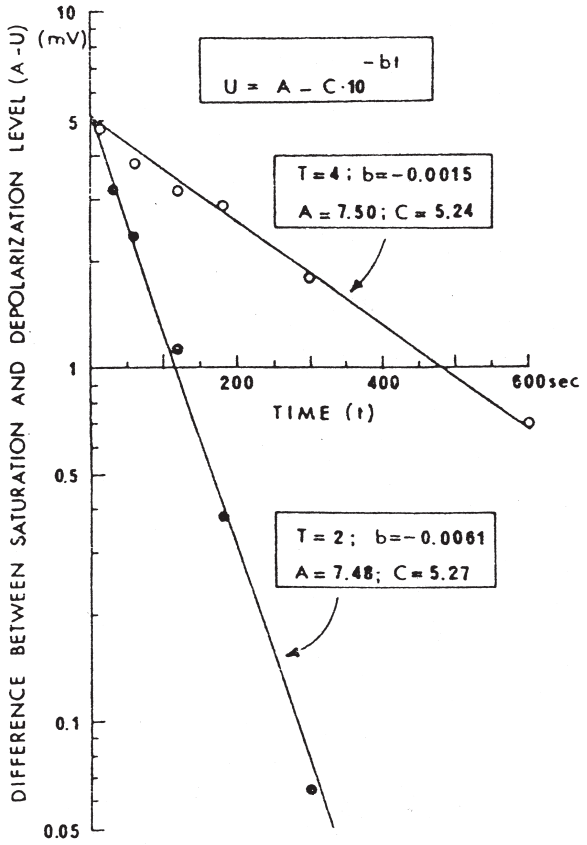

Fig. 4. Amplitude of light-evoked depolarization during adaptation in darkness of a visceral ganglion neuron. Abscissas: time (*t*) in darkness, ordinates: difference between initial depolarization amplitude (*A*) and amplitude of depolarization (*U*) obtained at a given moment t, when a short light flash was applied. T is the duration of test illumination in minutes. C is amplitude of depolarization when $t = 0$ and b is the rate constant. Note: $C = A - U$.

the same duration obtained in several experiments are presented in (Fig. 3) (curves a, b, and c). It can be seen that the action potential frequency reached a maximum 5-15 s after the onset of light. After that, although the action potential frequency occasionally decreased, it still remained significantly higher at every moment during illumination compared to the subsequent dark periods. That was the case not only in experiments done on the cell in the left parietal ganglion, where 1 min light/dark periods were induced (curve a), but also in experiments on the other two cells, where the illumination and the subsequent dark periods lasted 2 min (curves b and c).

Using the averaging and least square methods (R i s t a n o v i ć and P a š i ć , 1975) it was found that during the entire illumination period the action potential frequency of depolarized cells remained significantly higher than during the subsequent dark periods. Photosensitive neurons apparently behave as a slowly adapting receptor. However, with the light intensities used in the present experiments, possible dynamic and static components of the reaction could not be distinguished, although we observed that after the initial increment, the action potential frequency first decreased slightly and only after that did stabilization occur.

The depolarization and increment of action potential frequency, recorded in three neurons appear to be similar to the depolarizing generation potential recorded in many invertebrate photoreceptor cells, while in the most cases vertebrate cones and rods react to the onset of light with hyperpolarization (P r o s s e r , 1973). The photosensitive neurons in *Helix pomatia* ganglia offer the possibility of investigating both types of reaction to illumination. The membrane conductivity changes during light evoked depolarization and hyperpolarization of *Helix pomatia* neurons remain to be elucidated, together with potential differences between pigment contents of the two cell categories.

Reactivity of depolarizing neurons to light was also tested in the course of the dark period following a test illumination of 2 and 4 min. After the light was switched off, 10-s flashes of identical intensities were applied and depolarization recorded in a neuron hyperpolarized 10 mV below the resting membrane potential level. The data obtained in this experiment on a visceral ganglion cell are presented in Fig. 4. It can be seen that 300 s after a 2-min illumination period (T), the amplitude of light-evoked depolarization differed from the initial depolarization (i.e., from A=7.40 mV) by 0.1 mV. The recovery of the depolarization amplitude to its initial level after a 4-min illumination was much slower, the same level of depolarization being reached after 1150 s. The rate constant (b) representing the slope of the regression line is in this case more than four times higher than in the former one. However, in both cases parameter C remains nearly the same, which means that both graphs in (Fig. 4) start from the same value. The maximum reaction to light of the cell (A) was also almost the same. The fitting of the experimental points to straight lines is very good, as the relative standard deviations are in both cases less than 3 %. This also means that dark adaptation of the cell followed a logarithmic trend.

The light-induced current in photosensitive neurons

The photoresponse of an extraocular photoreceptor,

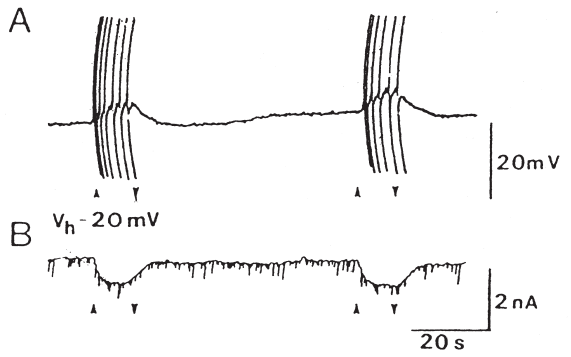

Fig. 5. Effect of light on a *Helix pomatia* photosensitive neuron in the left parietal ganglion. (A) Light-induced depolarization recorded under current-clamp. (B) Voltage-clamp record of the light-elicited current. Light on and off at arrows.

the photosensitive A-P-1 neuron in the abdominal ganglion of *Onchidium verruculatum*, was studied using a voltage-clamp with two microelectrodes (G o t o w , 1989). When the A-P-1 was voltage-clamped at the resting membrane potential, light induced a slowly developing inward current, which peaked at about 20 s. A decrease in membrane conductance accompanied this light-induced current, which corresponded to the depolarizing photoreceptor potential in the unclamped A-P-1 neuron. The steady-state light-induced current was a non-linear function of the membrane potential. The current-voltage relationship for the instantaneous light-induced current was almost linear. These voltage- and time-dependent properties of light-induced current were also observed in the photoreceptors of *Balanus* (B r o w n *et al.* 1970). However, the light-induced current in the solitary rods of salamander was voltage-dependent but not time-dependent (B a d e r *et al.* 1979), and in the extraocular photoreceptors of *Aplysia*, it was neither voltage- nor time-dependent (A n d r e s e n and B r o w n , 1979).

As described earlier (P a š i ć *et al.* 1977), in most *Helix pomatia* photosensitive neurons in the subesophageal ganglionic complex, the onset of light induces a slow depolarization. This is also the case with the photosensitive neurons in the left parietal ganglion. The onset of light induces a membrane depolarization recorded under current-clamp and an inward current shift under voltage-clamp configuration (Fig. 5).

At the level of the resting membrane potential (-40 to -50 mV), the maximum amplitude of the light-induced current was reached 10-15 s after the onset of light and ranged between 0.5 and 2 nA. During continuous illumination, the amplitude of the current declined slowly in the following 10-15 s to about 70 % of its initial value and remained unchanged until the end of the light stimulus.

Contrary to the light-induced depolarization, which can be accompanied by decreased membrane resistance ascribed to an increment of Na^+ conductance (P a š i ć and K a r t e l i j a , 1979), in the present experiments the inward current induced by light was associated with a 35 % decrement of slope conductance at membrane potentials more negative than about -10 mV (P a š i ć and K a r t e l i j a , 1995).

Light-induced current (IL) was recorded at different holding potentials. In some experiments, we applied several voltage steps and recorded the light-induced current. In other experiments, the voltage dependence of current was determined by applying a voltage ramp from –110 to 0 mV in 10 ms, in darkness and 15 s after the onset of light. The I_L value was obtained by subtracting $I\text{-}V_{light}$ from $I\text{-}V_{dark}$ (Fig. 6).

As seen from the recordings (Fig. 6A) and from the I-V curve (Fig. 6B), the light induced current decreased with hyperpolarization. In the experiment with a steady voltage-clamp, the reversal of I_L could not precisely be recorded, but at membrane potentials between –70 and –80 mV the current declined to zero. From the quasi-stationary *I-V* curve obtained by ramp-clamp, the reversal potential (E_{rev}) of I_L was determined and was found to range between –70 and –90 mV, which is close to the value of E_{eqv} for potassium ions in *Helix pomatia* neurons suggested by K o s t y u k (1968).

In the next experiments, the extracellular concentration was altered and the reversal potential for I_L was determined (Fig. 7). The graph presenting the mean results of three experiments depicted in (Fig. 8) reveals that the slope for 10-fold change of $[K^+]_o$ is 59.7 mV, which corresponds to that expected according to the Nernst equation, assuming an intracellular K^+ concentration of 110.9 mM (N e š i ć and P a š i ć , 1992). The assumption that I_L is due to the suppression of K^+ conductance is also based on the finding that in zero Na^+ and zero Cl^- solution the current remained unchanged.

Our results concerning the ionic mechanisms of the I_L recorded in *Helix pomatia* photosensitive neurons correspond to those obtained by G o t o w (1986), who ascribed the light-induced current in identified photosensitive neurons of *Onchidium verraculatum* to a decrement

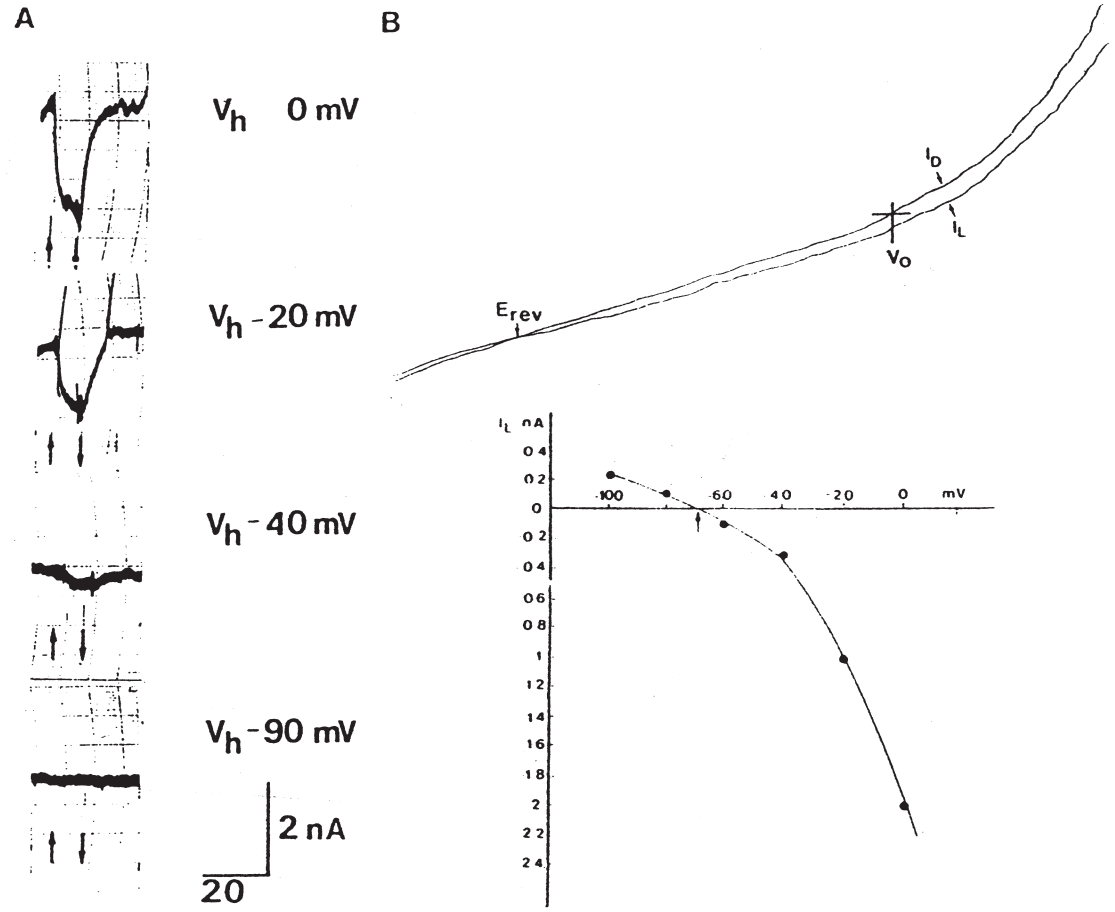

Fig. 6. The effect of membrane potential on the light-induced current. A) Current elicited by light at several holding potentials (V_h). B) Quasi-stationary *I-V* relation for the light-induced current obtained by slow voltage ramps. The *I-V* relation was calculated by establishing the difference between the ramp-generated plots shown in the upper parts of the figure. I_D, passive membrane current in darkness; *IL,* current elicited by light; V_0 , resting membrane potential; E_{rev}, reversal potential.

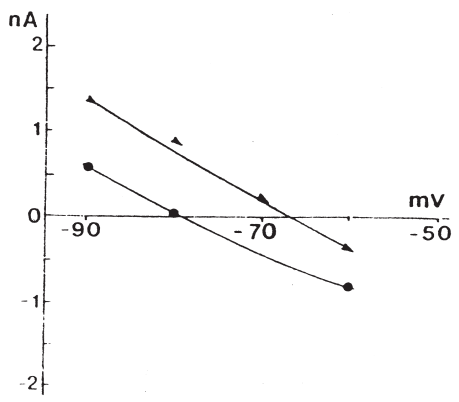

Fig. 7. Influence of increased $[K^+]_o$ concentration on the current response to light. Light-evoked inward current is plotted against holding potential in regular snail solution (•) and after the concentration of potassium was increased to 8 mM (►).

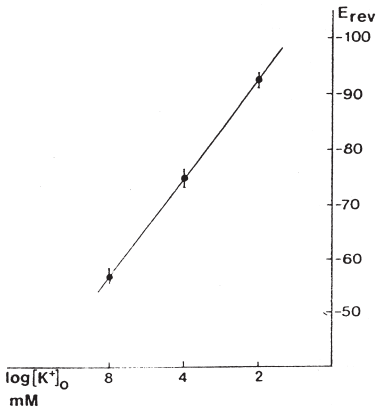

Fig. 8. Mean values of the reversal potential (E_{rev}) of I_L at three concentrations of K^+ ($[K^+]_o$) established from ramp-generated plots. The line has a slope of 59.7 mV per 10-fold change in $[K^+]_o$.

of potassium conductance. However, according to our earlier results, as well as results of other investigations, the responses to light of different photosensitive neurons in gastropod ganglia are diverse, as are the membrane mechanisms involved. The inhibitory reaction to light of the R2 and VPN photosensitive neurons in *Aplysia* was found to depend on increased potassium conductance, probably on the Ca^{2+}-dependent K^+ channel (Brown and Brown, 1973; Andresen and Brown, 1979). In previous experiments, neurons in the right parietal ganglion of *Helix pomatia* were shown to have a similar ionic mechanism of the light-induced hyperpolarization (Pašić *et al.* 1977). However, in a photosensitive neuron located in the left parietal ganglion, neuron C (Pašić and Kartelija, 1979), light induced a depolarization, which was ascribed to increased permeability to Na^+ ions.

Effect of cyclic GMP on the light-induced current

The depolarization of neurons caused by decrease in potassium conductance can be induced by various neurotransmitters and modulators, often involving second messengers (Kaczmarek and Levitan, 1987; Crouzy *et al.* 2001). They can affect only one type of the K^+ channels or they can affect different classes of them (Thompson, 1977; Hermann and Gorman, 1981a, 1981b; Schulz and Speckman, 1982). The modulation of a potassium channel, the S-K channel, in *Aplysia* neurons by serotonine FMRF-amide is among well described examples (Siegelbaum *et al.* 1982; Shuster *et al.* 1985; Brezina *et al.* 1987). The nature of the potassium channel was not investigated in the present work. However, our preliminary results show that the light-evoked current is relatively insensitive to extracellular tetraethylammonium chloride (TEA) and nearly totally suppressed by Ba^{2+}. Together with its relative voltage insensitivity, our data indicate that the potassium channel suppressed by light may share some characteristics with the S-K channel. However, while modulation of the S-K channel involves adenosine 3', 5'-cyclic monophosphate (cAMP) (Siegelbaum *et al.* 1982), the potassium channel involved here is modulated by guanosine 3',5'-cyclic monophosphate (cGMP).

Gotow and Nishi (1991) examined the internal messengers mediating the photocurrent of the A-P-1 molluskan extraocular photoreceptor. Injection of cGMP into the A-P-1 neuron produced an outward current associated with an increase in conductance. The steady-state *I-V* curve for the cGMP-induced current was non-linear. The steady state and instantaneous *I-V* curve for cGMP-induced current indicated that the internal cGMP induced a voltage- and time-dependent K^+ current. Their previous works (Gotow, 1989; Nishi and Gotow, 1989, 1998) showed that the photocurrent response of the A-P-1 neuron results from suppression of voltage- and time-dependent K^+ current by light. On the other hand, Gotow and Nishi (1991) demonstrated that the photocurrent was amplified by prior injection of inositol 1,4,5-trisphosphate (IP3). These results suggest that the cGMP-induced (dark) current is mediated by cGMP, and that its hydrolysis is then amplified by another messenger, IP3.

To explain the involvement of cAMP and IP_3 in the A-P-1 photoresponse, Gotow and Nishi (1991) postulate the existence of a parallel cGMP/IP_3 cascade model in phototransduction analogous to the cGMP cascade in vertebrate and invertebrate photoreceptors (Fein and Cavar, 2000; Ukhanov and Walz, 2001; Walz *et al.* 2000 a, b).

In our experiments we investigated the effect of light and cyclic GMP on identified photosensitive neurons in the left parietal ganglion of *Helix pomatia*. These neurons were chosen because in our previous experiments we found that the onset of light, besides inducing an inward current shift probably due to suppression of K^+ conductance, also causes broadening of the action potential in these cells by enhancing the voltage-dependent Ca^{2+} current (Pašić *et al.* 1987; Pašić and Kartelija, 1988, 1990). In addition, the phosphodiesterase inhibitor 3-isobutyl-1-methylxantine (IBMX) mimics the effect of light on Ca^{2+} current (Fig. 9), suggesting that one of the cyclic nucleotides increased by light could mediate the effect of illumination on the Ca^{2+} current.

Next, we tested the effect of a membrane-permeable cGMP analog, 8-bromoguanosine 3', and 5'-cyclic monophosphate (8-Br-cGMP), on the light-induced current. In all 100 cells examined, 8-Br-cGMP applied by itself mimicked the effect of light: it produced an inward current shift associated with a 28 % decrement of slope conductance (Fig. 10). In addition, in the presence of 0.1 mM 8-Br-cGMP, the maximum amplitude of the light-induced current was enhanced by 25 % (Fig. 11).

When cGMP was injected iontophoretically into the photosensitive neuron, it too mimicked the effect of light: an inward current was recorded with decreased slope conductance (Fig. 12).

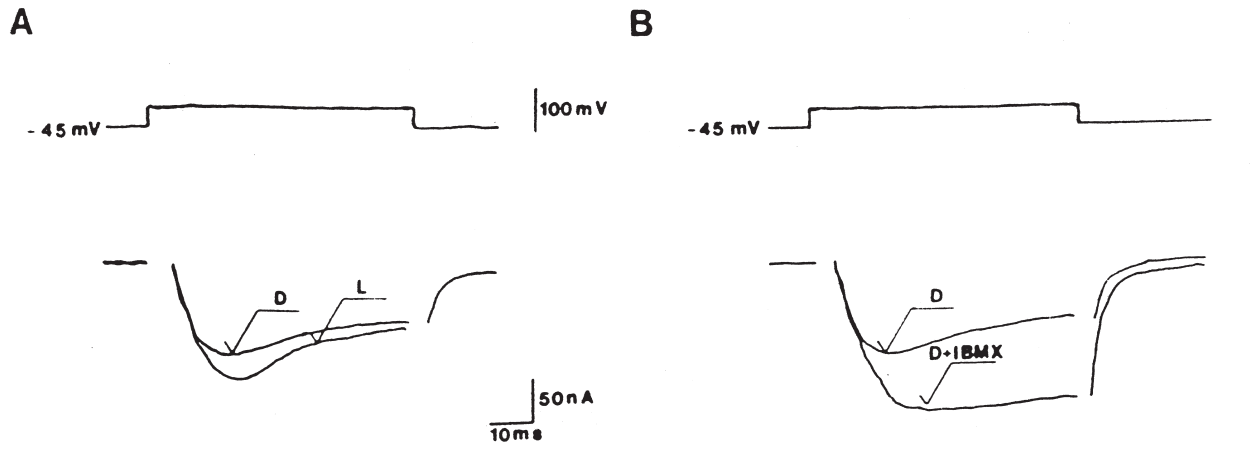

Fig. 9. Effect of light on a photosensitive neuron. A) Inward current recorded before (D) and after the onset of light (L). The cell was held at –45 mV and depolarized to 0 mV with a 60-ms pulse and was bathed in 10 mM Ba^{2+}, 80 mM TRIS, and 40 mM TEA. B) Inward current recorded in a photosensitive neuron bathed in the same solution as in A. Application of 0.1 mM IBMX evoked an increase in amplitude of the peak of inward current

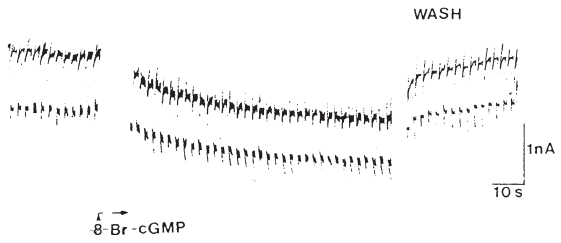

Fig. 10. Effect of 8-Br-cGMP on a photosensitive neuron. Arrows indicate the introduction of a cyclic GMP analog into the bathing solution. The recording was discontinued 5 min after adding the analog and before the start of washing.

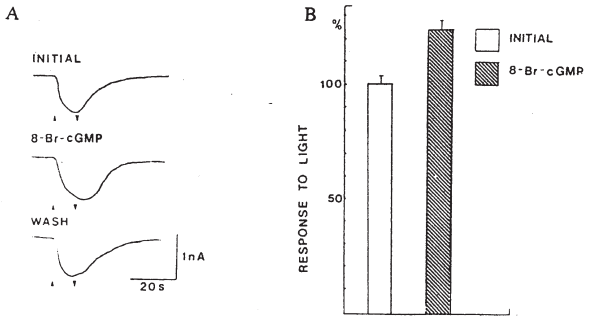

Fig. 11. Effect of 8-Br-cGMP on amplitude of the light-elicited current. (A) Recordings of the current in regular snail solution, 5 min after introducing cyclic nucleotide analog into the bathing solution, and 10 min after washing. (B) Mean percentage increment of maximum amplitude of light-elicited current before (100%) and 5 min after perfusion with 8-Br-cGMP-containing solution (n=5).

The *I-V* relationship of the responses induced by light and by 8-Br-cGMP application was compared (Fig. 13). We applied voltage-ramp from –100 to 0 mV in darkness and 15 s after the onset of light (Fig. 13A). The *I-V* curves were also obtained in darkness in the absence of 8-Br-cGMP and 5 min after it was added to the bathing solution (Fig. 13B). Our data show that the current induced by light and that induced by 8-Br-cGMP follow a similar course. In both cases the current decreases with hyperpolarization and E_{rev} is at about the same membrane potential, as can be seen in (Fig. 13C). In four other cells examined, E_{rev} for I_L and for $I_{8\text{-Br-cGMP}}$ was at about the same membrane potential and ranged between –75 and –80 mV.

It can therefore be assumed that both light and cGMP suppress the same conductance. The presented experiments suggest that the suppression of the potassium conductance evoked by application of light on these types of photosensitive neurons is mediated by cGMP. This assumption is supported by the finding that elevation of intracellular concentration of cyclic nucleotide either by adding its membrane permeable analog into the perfusion solution or by injecting it into the cell mimics the effect of light: in the dark, cGMP induces an inward current shift with decreased membrane conductance. Also, the *I-V* relation of I_L and I_{cGMP} follows a similar course and both currents have common E_{rev}. The similarity between the conductance change induced by external signals (e.g., a neurotransmitter) and a supposed intracellular mediator usually suggests that the presumed intracellular mediator is involved in the signaling pathway (K a c z m a r e k and L e v i t a n , 1987).

The enhancement of maximum amplitude of the

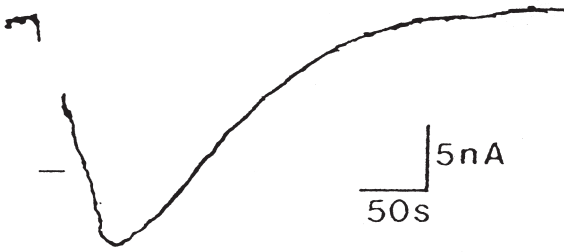

Fig. 12. Effect of iontophoretic injection of cGMP into photosensitive neuron. The effect of cGMP on the photosensitive neuron was tested by iontophoresis with 0.5 mM cGMP. CGMP was applied using a model 160 WPI microelectrode programmer. Negative pulses of 500 nA, lasting 30 s, were used for ejection of the cGMP.

light-induced current in the presence of 8-Br-cGMP observed in our experiments could be considered to be in contrast to findings in which occlusion of the reaction to neurotransmitters (e.g., serotonin) occurs after increasing the intracellular concentration of the second messenger (e.g., cAMP) (Paupardin-Tritch *et al.* 1985; Walsh and Byrne, 1985). The occlusion is taken as proof that the neurotransmitter and the second messenger act through a common mechanism. However, it should be considered that the intensity of light which we used to illuminate the neurons was not saturating and that the concentration of 8-Br-cGMP was relatively low (0.1 mM), 10 times lower than in the above quoted experiments. The same study also demonstrated that a moderate increment of the intracellular cAMP concentration augments the cell reaction to a neurotransmitter (e.g., serotonin) (Deterre *et al.* 1981; Walsh and Byrne, 1985). Indeed, in some of our experiments in which the light-induced current was recorded in the presence of a relatively high concentration (1 mM) of the phosphodiesterase inhibitor IBMX, the light-evoked current was significantly attenuated.

It was shown in the study of Gotow and Nishi (1991) that cGMP mediates a light response in *Onchidium verruculatum* photosensitive neurons. However, unlike our experiments on *Helix* neurons, in neurons of *Onchidium verruculatum* an injection of cGMP during darkness produced an outward current, which was suppressed by light. This evidence suggests that in the *Onchidium* neuron, light activates a phosphodiesterase, which, like vertebrate photoreceptors, reduces cGMP.

In view of our data indicating that the response to light of various photosensitive neurons is diverse, an opposite role of cGMP in phototransduction in different cells in two species is not unexpected. In some invertebrate photoreceptors, light is presumed to increase cGMP

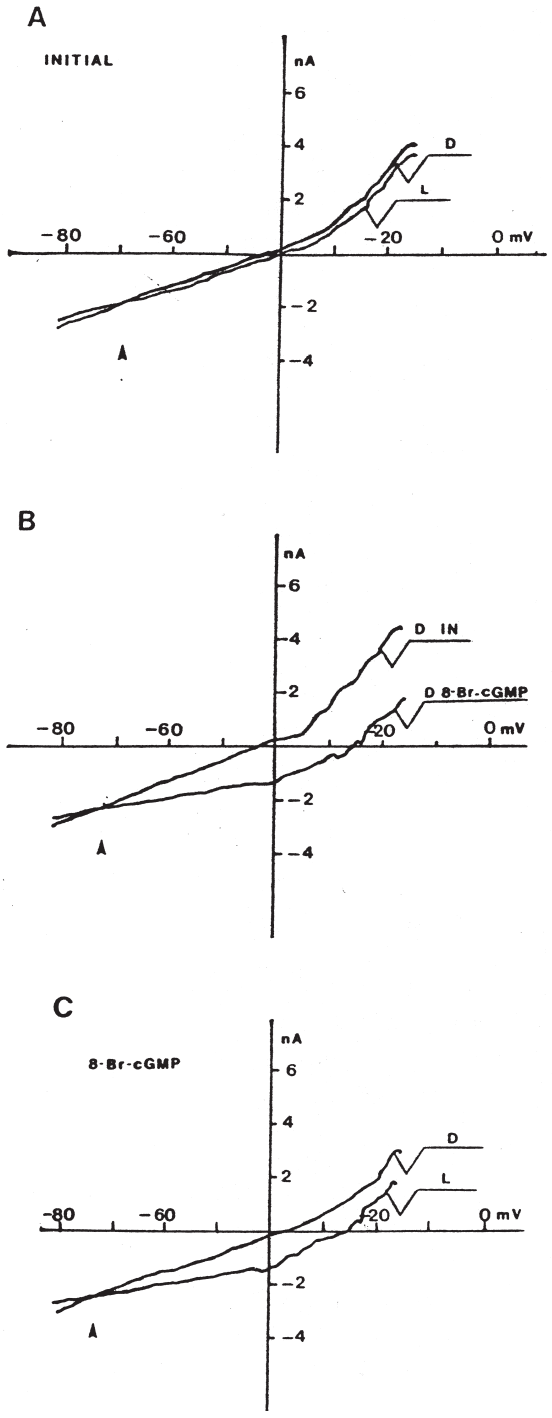

Fig. 13. Quasi-stationary *I-V* plots obtained by using voltage ramps. The membrane potential of the photosensitive cell was swept between –100 mV and 0 mV during 10 s. A) *I-V* relationship in regular snail solution before (D) and after onset of light (L). B) *I-V* plot in regular snail solution and 5 min after adding 8-Br-cGMP (0.1mM). C) *I-V* plot in snail solution containing 8-Br-cGMP before and after the onset of light.

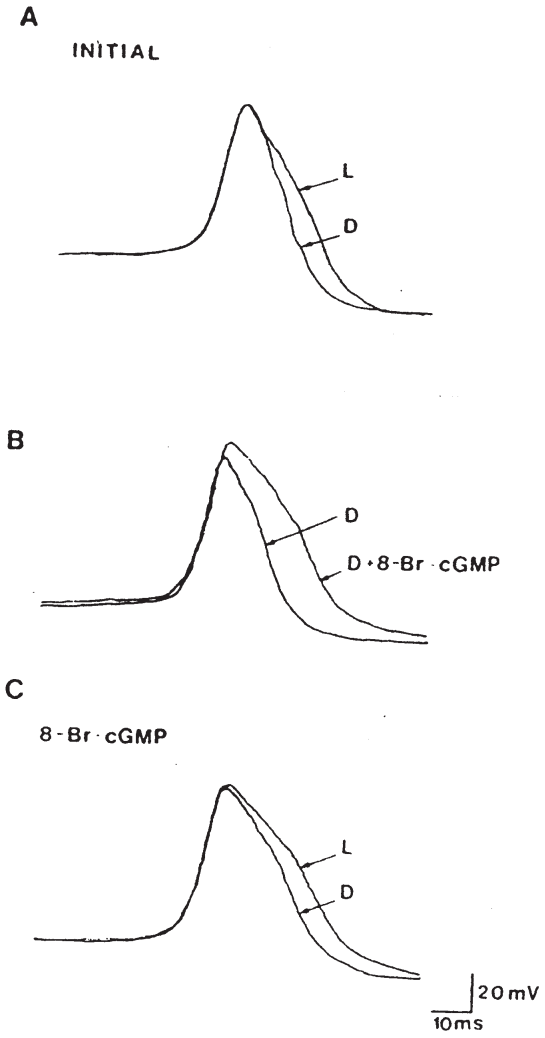

Fig. 14. Influence of light and 8-Br-cGMP (0.1mM) on the duration of action potentials of photosensitive neurons. (A) Action potentials evoked in darkness (D) and 15 s after the onset of light (L). B) Action potentials in darkness in the absence (D) and in the presence of 8-Br-cGMP in the bathing solution (D+8-Br-cGMP). C) Switching on of light in the presence of 8-Br-cGMP induces further broadening of the action potential.

(Pašić *et al.* 1977; Johanson *et al.* 1986; Wolken, 1988; Pašić and Kartelija 1990; Bacigulapo *et al.* 1991; Feng *et al.* 1991; Pašić and Kartelija 1991; Prosser, 1991), but it also increases cationic conductance. It thus seems that phototransduction in the left parietal ganglion neurons of *Helix* may differ in some respect from that in invertebrate or vertebrate photoreceptors. Whether phototransduction in *Helix* or other snail neurons or other extraocular photoreceptors involves a similar or different mechanism of phototransduction to the one described remains to be resolved.

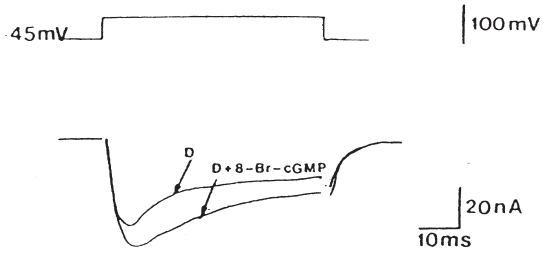

Fig. 15. Effect of 8-Br-cGMP (0.1mM) on the calcium current of photosensitive neurons. Inward current recorded in Ba^{2+}/ TRIS / TEA / 4-AP solution in darkness (D), before, and 10 min after adding 8-Br-cGMP to the bathing solution (D+8-Br-cGMP). The cell was held at –40 mV and depolarized to 0 with a 60-ms pulse.

Effect of cyclic GMP on the Ca^{2+} inward current

The photoresponse in some invertebrate photoreceptors is mediated either by cGMP or by Ca^{2+}. In one scheme, intracellular cGMP is increased by light, and the light-induced cGMP increase leads to generation of the receptor potential (Saibil, 1984; Johanson *et al.* 1986). In another scheme, internal Ca^{2+} is released by light to mediate the same receptor potential (Fein, 1986). Thus, the phototransduction mechanism of the A-P-1 neuron as well as its conductance mechanism are similar to those of vertebrate photoreceptors rather than invertebrate ones.

Involvement of cGMP in the response of photosensitive neurons to light is also supported by experiments comparing the effect of light and 8-Br-cGMP on the action potential and Ca^{2+} current. As seen in (Fig. 14), introduction of 8-Br-cGMP into the bathing solution during darkness increases the duration of the action potential by 40 %, as does the onset of light (Pašić and Kartelija, 1990). If light is switched on in the presence of 8-Br-cGMP, the action potential is broadened even further (by an additional 8 %).

To ascertain whether the 8-Br-cGMP-induced broadening of the action potential is mediated by enhancement of the Ca^{2+} current, we suppressed the Na^{+} and K^{+} currents by replacing sodium with hydroxymethyl-aminomethane (Tris) and by blocking the K^{+} currents with TEA, 4-AP, and Ba^{2+}. The light-induced current caused by suppression of potassium conductance was blocked (not shown), but illumination of the cell induced an increment of the Ca^{2+} inward current as did the application of 8-Br-cGMP (Fig. 15). From the ramp-generated *I-V* curves in the absence and presence of 8-Br-cGMP (Fig. 16), it can be seen that

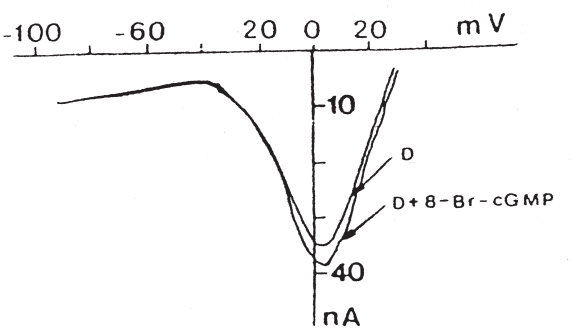

Fig. 16. Ramp-generated *I-V* curve from –100 mV holding potential in Ba^{2+}/ TRIS / TEA / 4-AP solution in the absence of 8-Br-cGMP and 10 min after it was adding to the bathing solution/dark.

the increment of Ca^{2+} current is induced at levels of membrane potentials between about –20 and +30 mV. The increment of the current ranged between 5 and 10 %, which is similar to the percentage augmentation of Ca^{2+} current induced by light (Pašić and Kartelija, 1990). Thus, our data suggest that the light-induced elevation of cGMP mediates the effect of light on the voltage-dependent calcium current.

The involvement of cGMP in Ca^{2+} current modulation was found in mammalian myocardium, where it inhibits the current (Wahler *et al,* 1990). Enhancement of Ca^{2+} current in *Helix* neurons induced by serotonin and mediated by cGMP has been described by Paupardin–Tritsch *et al.* (1986a). There is convincing evidence indicating that cGMP increases the Ca^{2+} conductance by activation of cGMP-dependent protein kinase (Paupardin–Tritsch *et al.* 1986b). In the present work, we did not address questions concerning the mechanism by which cGMP modulates the current involved in the reaction of neurons to light. The possible role of cGMP-dependent protein kinase in light-induced suppression of the potassium current and increment of the Ca^{2+} current remains to be resolved. Investigations on the role of other internal messengers in the reaction to light of photosensitive neurons could also be considered.

CONCLUSION

The physiological role of photosensitive neurons in central ganglia of snails is presently unknown. However, during full body extension outside the shell, there is enough light passing through the skin to excite or inhibit neurons in the central ganglia. Modification of voltage-gated currents (e.g., the Ca^{2+} current) by light could be of physiological significance, as it could modify synaptic transmission and therefore affect the animal's reaction to environmental factors. The importance of light in the life of *Helix aspersa* has been demonstrated by recent work dealing with the effect of the light regime as well as color on growth and sexual maturation of these snails (Bonnefoy–Claudet and Laurent, 1987).

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ФОТОСЕНЗИТИВНИ НЕУРОНИ КОД МЕКУШАЦА

ГОРДАНА КАРТЕЛИЈА[1], М. НЕДЕЉКОВИЋ[1] и ЛИДИЈА РАДЕНОВИЋ[1]

[1]Лабораторија за целуларну неурофизиологију, Институт за биолошка истраживања "Синиша Станковић", 11000 Београд, Србија и Црна Гора; *[2]Институт за физиологију и биохемију, Биолошки факултет*, *Универзитет у Београду*, 11000 Београд, Србија и Црна Гора

Уз познате фоторецепторе неки бескичмењаци имају једноставне екстраокуларне фоторецепторе. На пример, централна ганглија мекушаца садржи фотосензитивне неуроне. Ови неурони локализовани на дорзалној страни ганглије и на основу електрофизиолошких карактеристика претпоставља се да поседују унутрашње фоторецепторе. Међутим, поред ока, трансдукција светла се одвија и у овим екстраокуларним фоторецепторима. У овом раду анализирали смо реактивност ових нервних ћелија на светло и објаснили механизам који лежи у његовој основи.