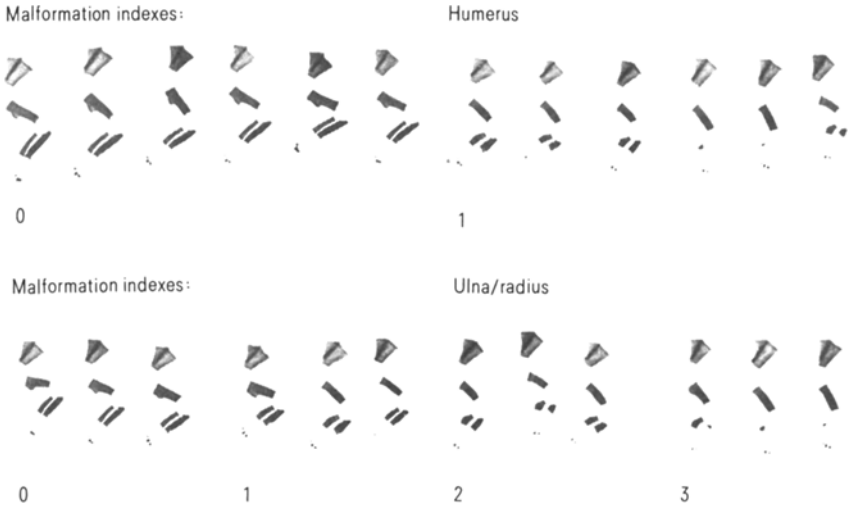

Figure 4. Forelimb malformations induced by retinoic acid. Pregnant female rats were intubated with retinoic acid (120 mg/kg) on day 13 of gestation. Fetuses were obtained by laparotomy on day 21 of gestation and processed for visualization of the skeleton with alizarin red S. Malformations of humeri and ulnae/radii were arbitrarily indexed 0–1 and 0–3 respectively, 0 representing controls.

Recently, Sporn and Roberts[12] reviewed the role of retinoids in differentiation and carcinogenesis and proposed that a molecular hypothesis for the mechanism of retinoid action that is compatible with the broadest range of experimental data is that retinoids modify gene expression. However, little information is available about how they control gene expression. The discovery of specific intracellular retinoid binding proteins[13,14] has led to the proposal that these binding proteins allow specific transfer of retinoids into the nucleus and to interactions with chromatin there.

Control of gene expression via interactions with protein kinases may integrate a receptor cascade system consisting of phospholipid degradation, Ca^{2+}, calmodulin, arachidonic acid, prostaglandins, cyclic AMP and cyclic GMP[2,3]. Biddulph et al.[15] reported changes in concentrations of prostaglandin E_2 and cyclic AMP during various stages of chondrogenesis in chick limb bud cell cultures, supporting a regulatory role for both prostaglandin E_2 and cyclic AMP in the early events associated with chondrogenesis. In this limb bud cell culture system retinoic acid not only inhibits chondrogenesis[16,17] but induces a time- and dose-dependent degradation of newly-differentiated cartilage nodules[17] similar to the degradation of cartilage in cultured fetal bones[5–7]. These effects of retinoids on cell differentiation, particularly in the processes of chondrogenesis, may be related to the mechanisms by which congenital limb defects are produced by retinoids. That the above receptor cascade system may be involved in retinoid action is supported by our findings that retinoic acid-induced cartilage degradation in vitro involves Ca^{2+} and calmodulin[8,18]. The present finding that the cyclohexanetrione Ro 31-0521, which stimulates prostaglandin synthesis, suppressed both cartilage degradation in vitro and teratogenicity in vivo induced by retinoic acid, further supports the view that the of action of retinoids on cell differentiation. In addition, it may help to elucidate the biological activity of cyclohexanetriones.

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Decrease of K^+ conductance underlying a depolarizing photoresponse of a molluscan extraocular photoreceptor

T. Gotow

Department of Physiology, School of Medicine, Kagoshima University, Kagoshima 890 (Japan), 3 April 1985

Summary. An identified neurone in the *Onchidium* abdominal ganglion responds to light with a depolarizing generator potential, so that this neurone functions as an extraocular photoreceptor. The light-evoked depolarizing response is produced by a selective decrease in K^+ conductance.

Key words. Molluscan extraocular photoreceptor; depolarizing photoresponse; decrease in K^+ permeability.

The hyperpolarizing photoresponse of most vertebrate ocular photoreceptors is produced by a decrease in membrane conductance to Na^+ ions[1]. This contrasts with the invertebrates studied to date, in which the hyperpolarizing or depolarizing response to light is associated with an increase in conductance to Na^+ and K^+ ions[2–8]. Thus, a photoreceptor potential produced by a de-

crease in membrane conductance was not thought to occur in any invertebrate photoreceptor system. However, I have found that an identified photosensitive neurone (extraocular photoreceptor) in the ganglion of the marine gastropod mollusc *Onchidium* responds to light with a graded depolarizing receptor potential which is due to a light-evoked decrease in K^+ conductance.

Materials and methods. The experiments were carried out on an identified neurone of the circumesophageal ganglion of *Onchidium verruculatum*. The photosensitive neurone identified as an extraocular photoreceptor in this study lies dorsally on the right upper quadrant of the abdominal ganglion, and has a relatively small spherical soma which is 80 µm or less in diameter. Thus, this neurone, designated as A-P-1, is distinct from the other giant photosensitive neurones (200–300 µm in diameter) which have been previously demonstrated in the same *Onchidium* ganglia[9,10].

The ganglia were placed in a 1 ml bath, and perfused with saline containing 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 10 mM tris-hydroxymethylaminomethane (THAM)-HCl adjusted to pH 7.8. Variations in external K^+ were made by adding or omitting KCl. Low Na^+ or Ca^{2+} solutions were made by replacing Na^+ or Ca^{2+} with THAM or Mg^{2+}, respectively. Isethionate salts were used as Cl^- substitutes. All solutions were kept at the same temperature of 21–23 °C. For intracellular recording, one or two single microelectrodes filled with 2.5 M KCl were inserted into the A-P-1. Current was applied to the cell through the second electrode, and a bridge circuit was used when a single electrode served both for applying current and for recording. White or monochromatic light from a tungsten quartz-iodine source was used for photostimulation. The intensity of light stimulus was controlled by neutral density filters and the radiant energy flux was measured at the level of the preparation with a radiometer.

Results and discussion. The resting potential of A-P-1 was −45 to −55 mV, and the input resistance at these potentials was 15–20 MΩ. Some properties of the photoresponse in an A-P-1 are shown in figure 1. White light stimulation, 30 s in duration, evoked a depolarizing generator (receptor) potential (fig. 1A). The amplitude of the depolarization was graded with the intensity of the light, and with brighter lights, spikes were superimposed on the graded depolarization (fig. 1B). The latency of onset of the light response was in the range of 300–500 ms, and usually it decreased with increasing light intensity. Membrane conductance changes during the photoresponse were studied by measuring the voltage drop produced by short (1s) hyperpolarizing constant current pulses. As shown in figure 1C, membrane conductance decreased during the depolarizing response to the same light stimulation as in figure 1A. In the dark, a passive depolarization of the membrane pre-set to nearly the peak value obtained during the photoresponse produced no significant changes in membrane input resistance (fig. 1D). Thus, the conductance decrease observed in association with the photoresponse is not due to membrane rectification.

The possibility that these photoresponses are due to light-evoked synaptic transmission through a presynaptic neurone was ruled out in the following ways. Exposure to the high Mg^{2+} saline containing 4 times the normal level of Mg^{2+}, which blocks synaptic transmission[11], had no significant effect on the response to light. Furthermore, the depolarization in response to light was maintained in the A-P-1 soma completely isolated from the ganglion by microdissection.

Monochromatic light also produced a slow depolarizing response in A-P-1 similar to that evoked by white light, and the waveforms of responses to any two monochromatic lights were identical when the light intensities were adjusted to give responses of equal amplitude. The spectral sensitivity (the reciprocal of the number of quanta at each wavelength which elicits a constant response), obtained from three experiments, had a peak at light of 490 nm in the range of 350 to 800 nm. These results suggest that a single intracellular photopigment is involved in the depolarizing photoresponse of A-P-1.

A series of experiments (figs 2 and 3) were performed to investigate the ionic species involved in the light-evoked membrane conductance decrease. When a constant light stimulus of 490 nm was presented at 5-min intervals to the dark-adapted A-P-1, a response of appropriate amplitude was reproducibly obtained under equal conditions of the resting potential and the external ionic composition. However, the light-evoked response was markedly altered by changing membrane potential levels by

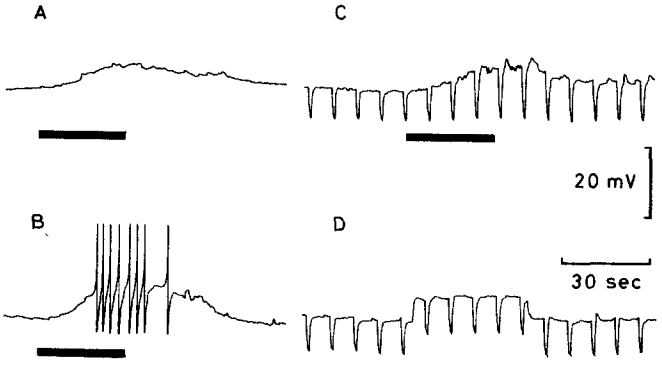

Figure 1. Depolarizing responses to white light of A-P-1. Light stimulus indicated by horizontal bar under each response was 30 s in duration, and its intensity was the same 0.9×10^{-3} W/cm^2 in A and C, and 1.8×10^{-3} W/cm^2 in B. Note that the generator potential was accompanied by spikes (whose top portions are cut) with increasing light intensity from A to B. In C, potential changes in response to 0.6 nA hyperpolarizing current pulses are shown before, during and after the photoresponse. In D, potential changes in the dark in response to the same hyperpolarizing current pulses were compared with those at passive depolarization by a sustained 0.3 nA current to nearly the same value reached during the photoresponse in C. All records are from the same A-P-1, which had initial resting potentials of −55 mV.

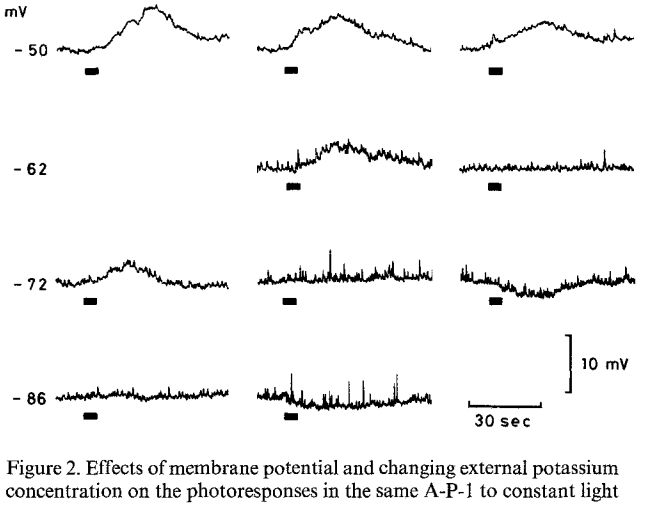

Figure 2. Effects of membrane potential and changing external potassium concentration on the photoresponses in the same A-P-1 to constant light stimuli of 490 nm. Potassium concentration in mM was 5 in column A, 10 (normal) in column B and 15 in column C. Membrane potentials indicated to the left in mV were pre-set by passing polarizing currents through one of two microelectrodes inserted into A-P-1. The reversal potantial of the photoresponse moved from its normal −72 mV level (B) to −86 mV (A), and to −62 mV (C). Bar under each trace shows light stimulus of 5 s in duration and 1.8×10^{-5} W/cm^2 in intensity at 490 nm. The initial resting potential in normal saline (B) was −55 mV.

passing currents across the membrane and by changing the concentration of potassium in the external solution. As shown in figure 2, the light-evoked potential became larger with depolarizing shifts, and smaller with hyperpolarizing shifts of the membrane potential. In normal (10 mM K^+) saline, the polarity of the light response was reversed at membrane potentials more negative than about −72 mV (fig. 2B). This reversal potential (−72 mV) in the normal saline was increased to approximately −86 mV when the external potassium concentration was halved (fig. 2A), and was decreased to approximately −62 mV when it was increased to 15 mM (fig. 2C). In figure 3, the results obtained from five experiments of this type are presented in graphic form, and the slope of shifts in reversal potential for a 4-fold change in external potassium shows 27 mV. This value reached roughly 80% of the theoretical slope value, 35 mV/4-fold change in external K^+, predicted by the Nernst equation.

Various ionic substitutions were made to test the contribution of other ions to the light response. Substitution of isethionate for external Cl^- had little effect on either the resting potential or the light response. Reduction of external Na^+ to 50 mM caused the resting potential to be hyperpolarized by 10–15 mV and reduced the amplitude of the light response. However, membrane polarization to the original resting potential by passing depolarizing currents returned the light response to the original level, indicating that reduction of the response by low Na^+ is due to passive hyperpolarization of the membrane. In addition, replacement of 10 mM Ca^{2+} with 20 mM Mg^{2+} did not effect the response.

These results suggest that the depolarizing receptor potential of A-P-1 is mainly produced by a decrease in K^+ conductance. In *Aplysia* extraocular photoreceptors, the light-evoked increase in K^+ conductance is elicited most effectively with 470–490 nm light[6,8]. Thus, it is interesting that a similar spectral sensitivity to light is associated with changes in K^+ conductance of opposite sign between *Onchidium* and *Aplysia* photoreceptors. In conclusion, this study is the first demonstration that a sensory receptor potential can be produced by a selective decrease in membrane K^+ conductance. This also shows that the photoresponse associated with a decrease in membrane conductance is not unique to the vertebrate photoreceptor.

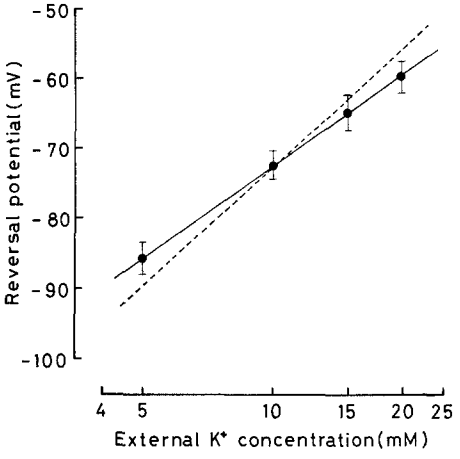

Figure 3. Relationship between the log of external potassium concentration and the reversal potential for the photoresponse of A-P-1. Each point was averaged from five A-P-1 neurones, with the SE of the mean shown by bars. The solid line drawn through each point shows 27 mV slope for 4-fold change in external potassium, and this slope value reached roughly 80% of the theoretical slope value (35 mV, dotted line) predicted by the Nernst equation.

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Cholinergic control of extracardiac pulsations in insects

K. Sláma

Institute of Entomology, Czechoslovak Academy of Sciences, Flemingovo 6, CS-16610 Praha (Czechoslovakia), 18 February 1985

Summary. The autonomic nerve system which controls extracardiac pulsations of hemolymph pressure appears to be quite susceptible to cholinesterase inhibitors (physostigmine, organophosphorus insecticides). In pupae of *Tenebrio molitor* L. and *Galleria mellonella* L., the treatment induced large peaks in hemolymph pressure (over 4 kPa). The peaks were repeated at more-or-less constant, 30-s intervals for several hours or days. Removal of the pupal brain neither affected extracardiac pulsations nor influenced the specific responses to the investigated drugs or insecticides.

Key words. Autonomic nerve system; anticholinesterase; physostigmine; epinephrine; acetylcholine; hemolymph pressure; *Tenebrio molitor; Galleria mellonella.*

The immobile developmental stages of insects exhibit occasionally rhythmic pulsations in mechanical pressure of the hemolymph[1]. The pulsations are produced by telescopic contractions of flexible abdominal segments. The control over frequency and amplitude of these extracardiac pulsations is achieved by an autonomic nerve system located in the thoracic ganglia of the ventral nerve cord[2]. According to certain physiological criteria (involvement in osmotic regulations and water balance, increased ventilation of respiratory gases, enhanced circulation of hemolymph through the appendages), the system can be considered analogous with the autonomic parasympathetic nerve system of the vertebrates. In this work I have used the extracardiac pulsations in pupae of the mealworm (*Tenebrio molitor* L.) and of greater wax-moth (*Galleria mellonella* L.) as a model system