Ionic Basis of Hyperpolarizing Receptor Potential in Scallop Eye: Increase in Permeability to Potassium Ions

Abstract. *Photoreceptors of the distal retina of the scallop respond to light with a large hyperpolarizing receptor potential which is caused by a selective increase in permeability to potassium ions.*

The depolarizing response of most invertebrate photoreceptors to light is produced by an increase in membrane conductance or permeability primarily to sodium ions (*1*), whereas the hyperpolarizing response of all vertebrate photoreceptors so far studied is associated with a decrease in membrane conductance (*2*). Evidence has accumulated that this hyperpolarizing response is also due to a change in permeability to Na^+ (*3*). This suggests that photoreceptor neurons have gates or channels in their membranes, controlled by light, which allow the passive movement of Na^+ down their electrochemical gradient. Thus, light acts either to increase permeability to Na^+ and produce a depolarizing receptor potential, or to decrease permeability to Na^+ and produce a hyperpolarizing receptor potential.

This generalization cannot explain the behavior of all photoreceptors, however, since visual cells in the chordate *Salpa* (*4*) and the mollusk *Pecten* (*5, 6*) have large hyperpolarizing responses to light which are associated with an increase in membrane conductance. The reversal potentials of these hyperpolarizing responses are in the range of −70 to −80 mv (*4, 6*), suggesting that the gating mechanism in visual receptors may involve a selective control over the movement of ions other than Na^+. In order to determine which ions control membrane potential in light and darkness, we have recorded intracellularly from hyperpolarizing photoreceptors under different extracellular ionic conditions (*7*).

The anatomy and physiology of the distal photoreceptors of the scallop *Pecten irradians* have been described previously (*5, 6*). Individual eyes were removed from the mantle and, after the cornea and lens were dissected away to expose the retina, the eye was held firmly in a narrow channel of a special chamber by suction (*8*). Artificial seawater flowed steadily and continuously through the channel. Changes in the perfusing solution were made by manually turning a valve located upstream from the retina, with about 15 seconds of dead time between switching the valve and the arrival of the new solution at the eye. Any resulting changes in membrane potential or response properties began at this time and were usually 90 percent complete within the next 30 seconds. Intracellular microelectrodes filled with 3*M* KCl (50 to 100 megohms resistance) and conventional d-c electronic equipment were used to record the membrane potential. The reference electrode was a Ag-AgCl wire connected to the bath through a 3*M* KCl agar bridge placed 1 cm downstream from the preparation. Changing the perfusing solution caused little (< 2 mv) or no change in junction potential between the microelectrode and reference electrode. The control solution was artificial seawater (ASW) (*9*). Chloride-free ASW was made by replacing Cl^- with propionate. Solutions of different K^+ concentration were made by substituting K^+ for Na^+. Sodium-free ASW was made by replacing Na^+ with either tris(hydroxymethyl)aminomethane (tris), choline, or lithium. All solutions were kept at the same temperature (22°C) and adjusted to have the same osmolarity and *p*H. The entire retina was evenly illuminated with flashes of white light of constant intensity, 100 msec in duration, presented at 10-second intervals. The light energy in the range 400 to 700 nm was approximately 10^{-4} watt/cm², which was sufficient to produce saturating receptor potential responses.

Figure 1A shows the response of a distal photoreceptor to a light flash in normal ASW and in Cl^--free ASW. Complete replacement of extracellular Cl^- with propionate had little effect on either the resting potential or the light response, indicating that the permeability to Cl^- is of little importance in determining the membrane potential or generating the receptor potential. The effects of removing extracellular K^+ were quite different: Fig. 1B shows the response of another photoreceptor in normal ASW and in K^+-free ASW. Although removing K^+ from the medium had little effect on the resting potential, the response to light became much larger. Increases in extracellular K^+ reduced both the resting potential and the response to light, but the effect was much greater on the light response than on the resting potential. In a few cases, it was possible to record from a single receptor long enough to make a number of changes in the external K^+ concentration. Figure 2 is a plot of the resting potential in darkness and the potential at the peak of the response to light against the external K^+ concentration. The potential was dependent on K^+ concentration to a much greater extent in light than in darkness. The points representing the membrane potential at the peak of the light response fall on a line with a slope of about 52 mv per tenfold change in external K^+, which is close to the theoretical

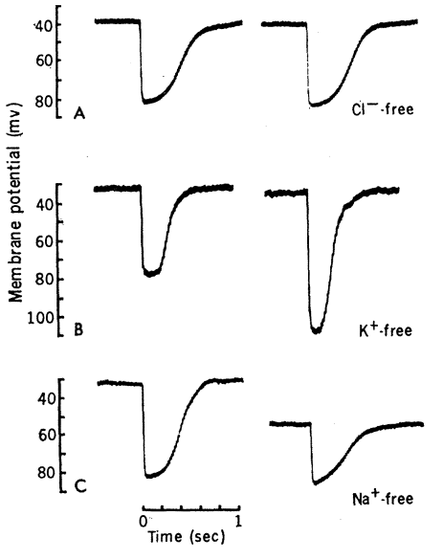

Fig. 1. Effect of ionic substitutions on membrane potential in light and darkness. Each pair of responses is from a different cell; the response on the left in each row is in normal ASW and that on the right is in (A) Cl^--free ASW, (B) K^+-free ASW, and (C) Na^+-free ASW (Na^+ replaced with choline).

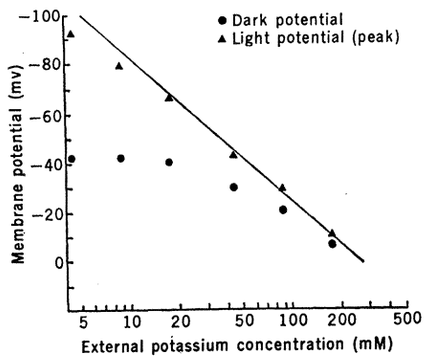

Fig. 2. Plot of membrane potential in darkness (circles) and at peak of receptor potential (triangles) as a function of external K^+ concentration. The straight line has the slope predicted by the Nernst equation for a K^+ electrode.

slope of 58 mv predicted by the Nernst equation for a K^+ electrode (shown by the continuous line in Fig. 2). Our results indicate that the receptor potential is mainly due to an increase in membrane permeability to K^+.

It is apparent from Fig. 2 that membrane potential in darkness is not solely determined by permeability to K^+. Figure 1C shows the effect of removing external Na^+ on the resting potential and light response. Replacement of Na^+ with tris, choline, or lithium caused the resting potential to become more hyperpolarized and reduced the amplitude of the light response. However, when saturating flashes were used the absolute potential reached by the peak of the light response was very little affected by complete removal of Na^+. The low value of the membrane potential in the dark and the large hyperpolarization produced by removal of external Na^+ suggest that permeability to Na^+ contributes significantly to resting potential in these cells; that is, compared with other neurons they have a relatively high ratio of Na^+ permeability to K^+ permeability.

It is of interest that a light-evoked hyperpolarization in a nonreceptor cell, the giant neuron of the *Aplysia* abdominal ganglion, is also produced by an increase in permeability to K^+ (*10*). This response has a very long latency and a slow time course, and is believed to result from absorption of light by intracellular pigments (*11*) of a different type than the membrane-bound pigments normally associated with photoreceptor cells. It is possible, however, that in both receptor and nonreceptor cells the effect of illumination is to release a similar intermediate product which initiates the permeability changes that occur in the plasma membrane. As yet the actual sequence of reactions leading to changes in membrane permeability has not been worked out for any photoreceptor system.

This is the first demonstration that a visual receptor potential can be produced by a selective increase in membrane permeability to K^+. In other sensory receptor cells, both visual and nonvisual, where the ionic basis of the response has been studied, the receptor potential has been attributed mainly to changes in Na^+ permeability. Our results, along with the recent finding that the hyperpolarizing mechanoreceptor potential in *Paramecium* is due to an increase in K^+ conductance (*12*), establish that the changes in ionic permeability which produce the receptor potential need not be specific for or even involve Na^+. It is likely that changes in K^+ permeability also underlie the receptor potential of other photoreceptors.

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Adenosine 3′,5′-Monophosphate: Inhibition of Complement-Mediated Cell Lysis

Abstract. *An increase in adenosine 3′,5′-monophosphate (cyclic AMP) in rat mast cells, achieved by stimulating the cells with prostaglandin E_1, by preventing cyclic AMP breakdown with aminophylline, or by adding exogenous dibutyryl cyclic AMP, prevented complement-mediated cytolysis as assessed by both histamine release and vital dye exclusion. Dibutyryl cyclic AMP also suppressed water-induced osmotic lysis.*

In contrast to the facilitory role of adenosine 3′,5′-monophosphate (cyclic AMP) as a "second messenger" initiating intracellular responses to extracellular stimuli, its role in the antigen-induced secretion of chemical mediators from human peripheral leukocytes, lung, and nasal polyps sensitized with immunoglobulin E (IgE) is suppressive (*1*). Its action in lung is preceded by a calcium-dependent activation of a serine esterase, an energy-dependent step, and a second calcium-dependent stage inhibitable by ethylenediaminetetraacetic acid (*2*). The inverse relationship between intracellular concentrations of cyclic AMP and mediator release has now been directly demonstrated by use of purified rat mast cells challenged with rabbit antiserum directed against rat Fab, a noncytolytic reversed anaphylactic reaction (*3*). Histamine release from rat mast cells may also be immunologically achieved by complement-dependent cytolytic mechanisms, and the role of cyclic AMP in modulating this reaction was investigated.

The interaction of mast cells with rabbit antiserum directed against mast cell–specific antigens forms stable sensitized cells that lyse after addition of normal serum as a source of complement (*4*). Pleural and peritoneal cells from male Sprague-Dawley and ACI rats (Gofmoor Farms, Westboro, Massachusetts) weighing 250 to 300 g were obtained (*5*), and the mast cells were studied either in mixed cell suspensions containing 5 to 10 percent mast cells or after isolation to 90 to 98 percent homogeneity by centrifugation into a solution of Ficoll (Pharmacia, Uppsala, Sweden) (*6*). Rabbit antiserum against rat mast cells (Rab anti-RMC) prepared as described was heated at 56°C for 30 minutes and dialyzed against Tyrode's solution (*4*). Interaction of Rab anti-RMC with either mixed cell suspensions or isolated mast cells—both suspended in Tyrode's 0.1 percent solution (Tyrode's gel) so as to contain 10^5 mast cells in 0.1 ml—for 15 minutes at 37°C, followed by washing, sensitized the cells such that the introduction of rabbit serum (10 percent by