Control of Membrane K^+ Permeability in a Hyperpolarizing Photoreceptor: Similar Effects of Light and Metabolic Inhibitors

Abstract. *In the hyperpolarizing photoreceptors of the scallop* Pecten irradians *the metabolic inhibitors cyanide and 2,4-dinitrophenol cause a rapid hyperpolarization and increase in membrane permeability to potassium ions, similar to the effect of light. Cellular metabolism appears important in maintaining the low permeability to potassium ions necessary to keep the membrane depolarized in darkness, possibly by regulating the intracellular calcium ion concentration.*

In photoreceptors, light triggers a change in membrane permeability which releases energy previously stored across the cell membrane in the form of ionic concentration gradients. The passive movement of ions down their concentration gradients and the resulting alterations of membrane potential provide a simple, but effective method of transducing information about the external world. In many photoreceptors it is primarily the permeability to Na^+ which is modulated by illumination. We have studied another class of photoreceptors, found in mollusks (*1*), in primitive chordates (*2*), and more recently by others in a vertebrate (*3*), which have large hyperpolarizing responses to light that are associated with an increase in membrane conductance. For this class of cells it is important to determine which ions produce the response and how light controls the movement of these ions.

The ionic basis of this type of response has so far been studied in only one example, the distal photoreceptor of the scallop (*4*). In these cells the hyperpolarizing receptor potential is generated by an increase in membrane permeability to potassium ions (P_K). In darkness the photoreceptor membrane has a relatively low permeability to K^+; in the presence of light P_K increases and membrane potential moves passively toward the K^+ equilibrium potential, which is considerably more negative than resting potential. Brown and Brown (*5*) have shown that the light-evoked hyperpolarization of a nonreceptor cell, the giant neuron of the *Aplysia* abdominal ganglion, is also produced by an increase in P_K. Their results show that increasing free intracellular Ca^{2+} increases P_K, thereby hyperpolarizing the neuronal membrane. In this report we show that, in the hyperpolarizing photoreceptors of the scallop eye, metabolic inhibitors which are likely to increase intracellular Ca^{2+} produce an increase in the membrane P_K.

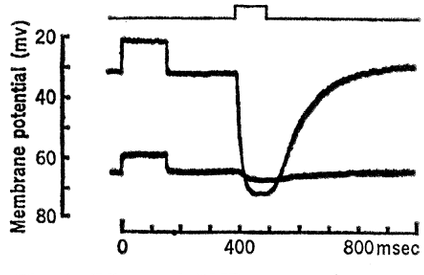

Fig. 1. Effects of DNP on membrane potential, conductance, and response to light. The upward deflection at the beginning of each trace is the membrane response to a current pulse of 1.5×10^{-10} amp. A reduction in response amplitude indicates an increase in membrane conductance. The downward deflection which follows is the receptor potential in response to a light flash whose duration is indicated on the horizontal line above. The upper trace is the response in normal ASW; the lower trace is the response of the same cell in the presence of 0.2 m*M* DNP in ASW.

The exposed retina of the scallop *Pecten irradians* was perfused with artificial seawater (ASW) (*6*) which could be rapidly exchanged with ASW solutions containing different metabolic inhibitors or ionic substitutions. The retina was stimulated with brief flashes of white light of constant intensity at 10-second intervals during the entire recording period. Details of the perfusion technique and intracellular recording methods have been given in an earlier report (*4*). A Wheatstone bridge circuit was used to measure changes in membrane conductance in the presence of various metabolic inhibitors. Four inhibitors were used to study the relation between cellular metabolism and membrane permeability: ouabain, a specific inhibitor of the Na pump; 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation; cyanide, a respiratory poison which interferes with electron transport; and iodoacetic acid (IAA), an inhibitor of glycolysis. All solutions were kept at the same temperature (22°C) and were adjusted to have the same *p*H. Because it was difficult to record from photoreceptors for long periods of time, exposure to inhibitors and to different ionic media was limited to the time necessary to make a reliable potential measurement once a stable membrane potential level had been reached. The maximum period of exposure to an inhibitor was approximately 3 minutes.

The dependence of a low membrane P_K in the dark on cellular metabolism is shown by the effects of DNP and cyanide on membrane potential and conductance. Figure 1 shows the effect of 0.2 m*M* DNP on a photoreceptor. Within 30 seconds after exposure to DNP, the cell underwent a large increase in membrane conductance and hyperpolarized by about 35 mv. These membrane changes produced by DNP are similar to those occurring in response to a bright stimulating light. Although the size of the receptor potential was greatly reduced, the absolute value reached by the peak of the response was only slightly affected by DNP. On return to normal ASW there was a rapid recovery of membrane potential and conductance (not shown). Similar results were obtained by perfusing the eye with 2 m*M* sodium cyanide. These effects, however, do not occur with all inhibitors. For example, up to 3 minutes' exposure to 5 m*M* IAA or 0.5 m*M* ouabain had no significant effect on membrane potential or light response.

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 in the presence of either DNP or cyanide. Figure 2 shows membrane potential in darkness and at the peak of the response to a saturating light flash as a function of the external K^+ concentration. Data are shown for the same cell in normal ASW and in the presence

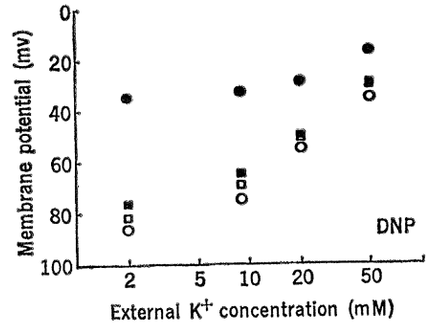

Fig. 2. Plot of membrane potential in darkness (closed symbols) and at the peak of the receptor potential (open symbols) as a function of external K^+ concentration. Data are shown for the same cell in normal ASW (circles) and in the presence of 0.2 m*M* DNP in ASW (squares).

of 0.2 m*M* DNP. In normal ASW membrane potential is dependent on the external K^+ concentration to a much greater extent in light than in darkness, as reported earlier (*4*). In the presence of DNP this disparity is greatly reduced, and membrane potentials both in the dark and in light show a strong dependence on the external K^+ concentration. The same kind of increased dependence of membrane potential in darkness on the external K^+ concentration was observed when 2 m*M* cyanide was used instead of DNP. We have not done experiments to determine whether the slight reduction in the potential reached by the peak of the light response in the presence of DNP or cyanide is due to a decrease in internal K^+ concentration or to interference with some earlier step in the transduction process.

The hyperpolarization of membrane potential, the increase in membrane conductance, and the increased responsiveness of the membrane to changes in the external K^+ concentration indicate that the P_K of the photoreceptor membrane increases in the presence of DNP or cyanide. We cannot completely exclude the possibility that these compounds have a direct effect on membrane permeability since DNP, but not cyanide, has been reported to increase the P_K of nerve cell membranes (*7*). However, the finding that both DNP and cyanide increase P_K in our cells makes an alternative hypothesis more likely.

The similarity of the effects of DNP and cyanide, and the lack of effect of ouabain or IAA, suggest that the observed permeability changes result from interference with the production of adenosine triphosphate (ATP) by oxidative phosphorylation. The small size and large surface-to-volume ratio of these photoreceptors make it likely that ATP stores are quickly depleted when DNP or cyanide are applied. An increase in intracellular Ca^{2+} concentration has been observed in other cells following exposure to either DNP or cyanide (*8*). This is to be expected since active uptake of Ca^{2+} from the cytoplasm by the mitochondria is an ATP-dependent process (*9*). In several types of cells there is evidence that increasing the intracellular Ca^{2+} concentration, either directly or by treatment with metabolic inhibitors (*5, 10*), causes an increase in P_K. We suggest, therefore, that the hyperpolarization and increase in K^+ conductance observed in the presence of DNP and cyanide is due to an increase in intracellular Ca^{2+} concentration. Since the receptor potential in these cells is also generated by an increase in P_K, it is possible that light-induced changes in intracellular Ca^{2+} concentration control P_K in those photoreceptors whose hyperpolarizing response is due to an increase in conductance (*11*).

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6. The composition of the ASW in millimoles per liter was Na^+, 425; K^+, 9; Ca^{2+}, 9; Mg^{2+}, 48; Cl^-, 496; SO_4^{2-}, 26; HCO_3^-, 2 [G. F. Cavanaugh, *Formulae and Methods* (Marine Biological Laboratory, Woods Hole, Mass., 1956), vol. 5, p. 55].
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11. It has been proposed that a light-induced increase in intracellular Ca^{2+} may cause the decrease in Na^+ permeability which generates the hyperpolarizing receptor potential found in most vertebrate photoreceptors [S. Yoshikami and W. A. Hagins, in *Biochemistry and Physiology of Visual Pigments*, H. Langer, Ed. (Springer-Verlag, Berlin, 1973), pp. 245–255; W. A. Hagins, *Ann. Rev. Biophys. Bioeng.* **1**, 131 (1972)].
12. Much of this work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts. Supported in part by NIH grant EY01157.

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Blepharmone: A Conjugation-Inducing Glycoprotein in the Ciliate Blepharisma

Abstract. *Gamone 1 of* Blepharisma intermedium *was isolated, identified as a slightly basic glycoprotein (molecular weight, 2×10^4), and designated as blepharmone. At the concentration of 6×10^{-8} milligram per milliliter, it specifically transforms mating type 2 cells, so that they can conjugate in about 2 hours.*

Conjugation of *Blepharisma intermedium* is induced by a few hours of interaction between cells of complementary mating types (*1, 2*). Type 1 cells excrete gamone 1, which transforms mating type 2 cells so that they can unite and, at the same time, induces them to produce and excrete gamone 2. This gamone in turn transforms type 1 cells so that they can unite, and it also promotes the production and excretion of gamone 1. Transformed cells can unite in all three possible combinations of mating types but only heterotypic pairs (1–2) complete conjugation. Homotypic pairs (1–1, 2–2) may persist for days, but the process of conjugation appears to stop at the stage of pair formation. Gamone 2, blepharismone (*3*), was isolated, identified as calcium-3-(2′-formylamino-5′-hydroxybenzoyl)lactate (*4*), and synthesized (*5*). Gamone 1 was suggested to be a protein with a molecular weight of about 2×10^4 (*2*). We have isolated and partially characterized this gamone, designated as blepharmone.

Mating type 1 cells were grown (*2*), concentrated, washed with SMB (1.5 m*M* NaCl, 0.05 m*M* KCl, 0.4 m*M* $CaCl_2$, 0.1 m*M* $MgSO_4$, 2×10^{-3} m*M* EDTA, 2 m*M* sodium phosphate buffer of *p*H 6.8), and suspended in SMB containing 32 units of gamone 2 per milliliter (*6*) and 0.01 percent albumin (bovine serum albumin, Behringwerke) at the density of 0.01 ml of packed cells per milliliter (about 10^4 cell/ml). Albumin was added to protect the gamone activity of blepharmone (*2*). After the suspension was kept for 1 day at 25°C, the cell-free fluid was obtained by removing cells by centrifugation. The cells were resuspended as described above, and the process was repeated for several days. The gamone 1 activity of the cell-free fluid was 0.8×10^4 to 12.8×10^4 unit/ml (*6, 8*); the cell-free fluid could be stored frozen for a few months without change in the activity.

The cell-free fluid (20 liters) having