Biophys. Struct. Mechanism 3, 135–138 (1977)

Biophysics of Structure and Mechanism

Some Considerations on the Ion Transport Properties of the Rod Disc Membrane*

Juan I. Korenbrot

Departments of Physiology and Biochemistry,
School of Medicine, University of California,
San Francisco, CA. 94143, USA

Abstract. The ion transport properties of the disc membranes in rod outer segments are discussed on the basis of available data. The properties of an air-water interface film of spectroscopically intact and chemically regenerable rhodopsin are presented, and results of studies of ion binding to these films are reported.

Key words: Rods – Disc membrane – Rhodopsin – Interface film – Ion binding.

The rod cell in the vertebrate retina hyperpolarizes in response to the absorption of light by rhodopsin. This hyperpolarization is the result of a decrease in the conductance to Na ions of the plasma membrane of the rod outer segment (Hagins, 1972; Montal and Korenbrot, 1976). Photoactivation of a single rhodopsin molecule reduces the Na current by as much as 1–3% (Hagins et al., 1970; Cone, 1973). Because of the large numerical gain in the photoresponse and because the rhodopsin containing discs are structurally, electrically and osmotically isolated from the plasma membrane, an "internal messenger" which would diffuse from the disc to the plasma membrane has been hypothesized. This internal messenger would couple the photoexcitation of the rhodopsin molecule to the change in current across the plasma membrane (Hagins, 1972; Cone, 1973). The observations that increases in the Ca ion concentration in the solution bathing the outer segment (Yoshikami and Hagins, 1973; Korenbrot and Cone, 1972; Brown and Pinto, 1974), and that even smaller increases in Ca concentration in the intracellular space (Hagins and Yoshikami, 1974; Wormington and Cone, 1975), mimic the electrophysiological effect of light led Hagins and Yoshikami (Yoshikami and Hagins, 1973; Hagins, 1972) to propose that Ca ions act as the internal messenger. In their view, light would cause release of Ca from the discs, increasing intracellular free Ca concentration and reducing Na current across the plasma membrane.

---

* Presented at the EMBO-Workshop on Transduction Mechanism of Photoreceptors, Jülich, Germany, October 4–8, 1976

Although elegant experiments have indeed shown that Ca ions mimic the effect of light, demonstration of light-dependent Ca release from discs has been less successful. Theoretical calculations by Cone (1973) and Hagins and Yoshikami (1975) suggest that a single excited rhodopsin molecule *in vivo* should cause the release of 20–500 Ca ions from a disc. Some reports of light-induced Ca release from discs have appeared (Hendricks et al., 1974; Hemminki, 1975; Liebman, 1974; Mason et al., 1974; Szut and Cone, 1974), but the reported stoichiometries have been in the 1–5 Ca per rhodopsin range and in no case have the kinetics of the release been studied. If the Ca hypothesis is indeed correct, why has there been such lack of success in demonstrating quantitatively Ca release from discs upon illumination? A possible explanation arises if we consider some biochemical properties of the disc membrane. Of the protein in the disc membrane, 85–90% is rhodopsin (Daemen, 1973) there is also a small amount of Mg ATPase (Ostwald and Heller, 1972) (about 700 molecules per disc in cattle) but essentially no ouabain sensitive Na-K ATPase (Frank et al., 1973; Zimmerman et al., 1976) (less than 5 molecules per disc in cattle). An expected consequence of these enzymatic characteristics would be that at equilibrium Ca, but not Na or K ions, would be actively transported. If we assume that the disc membrane, like the plasma membrane from which it is derived, is relatively more permeable to Na than to Ca ions then at equilibrium the disc membrane potential will be near zero if the Ca active transport is non-electrogenic. If the Ca active transport, on the other hand, were electrogenic the driving force on the Ca flux across the disc membrane could be dramatically different. If we remember that the intradisc volume is only of the order of 10^{-17} l, it is easy to see that under in vitro conditions tested so far, which have relied on intrinsic emfs for Ca, emf conditions could have been so changed as to produce the very small fluxes reported to date. It is also possible, of course, that release of Ca from the discs is not a transport phenomenon but release of Ca from some light-sensitive binding site. It is unlikely that such mechanism would provide the stoichiometry required in vivo. Furthermore, we show below that rhodopsin itself is not a light-sensitive Ca binding site.

The properties of rhodopsin as a possible light-sensitive binding site for Ca have been investigated in a novel model system. In this system all spectroscopic and chemical-reactivity characteristics of rhodopsin tested so far have been found to be identical to those in the disc membrane. The model system consists of a monomolecular film of rhodopsin molecules surrounded by a "skirt" of phospholipid organized at an air-water interface. Rhodopsin molecules isolated and purified from cattle retinas and surrounded by a bilayer of purified egg PC (phosphatidyl choline) are spread on the surface of water by a novel procedure. Surface tension measurements confirm the formation of a stable, insoluble film at the air-water interface. Electron-microscopic studies reveal that, at appropriate rhodopsin to phospholipid mole ratios, the interface films consist of uniformly distributed, non-overlapping fragments of lipid bilayer in which rhodopsin is present as the only protein. Spectrophotometric characteristics of the rhodopsin films have been studied in multilayers of the Blodgett-Langmuir type. In the dark, absorption spectra with a λ_{max} of 498 nm, typical of rhodopsin, are measured. Upon illumination of these films, the absorption spectrum can be seen to change through a sequence of colored intermediates. This photoreaction can be stopped at an intermediate with 480 nm λ_{max} (Meta I) by complete drying of the film. The 480 nm photointermediate can be photoreversed to 498 nm

(photo-regeneration). Rhodopsin at the interface is chemically regenerable. Bleached rhodopsin (opsin) can be spread over an aqueous subphase containing 11-cis retinal, and rhodopsin can be produced with over 80% efficiency. Surface binding of ^{45}Ca to the rhodopsin interface film has been measured with an end window gas flow counter. In the concentration range of 10^{-6} to 10^{-4} M/l $CaCl_2$ in the subphase, no effect of light on the Ca binding by rhodopsin has been detected up to levels of illumination which bleached over 45% of the rhodopsin molecules.

References

Brown, J. E., Pinto, L. H.: Ionic mechanism for the photoreceptor potential of the retina of Bufo marinus. J. Physiol. (Lond.) **236,** 575–591 (1974)

Cone, R. A.: The internal transmitter model for visual excitation. Some quantitative implication. In: Physiology and Biochemistry of the visual pigments (H. Langer, ed.). Berlin-Heidelberg-New York: Springer 1973

Frank, R. N., Cavanagh, H. D., Kenyon, K. R.: Light-stimulated phosphorylation of bovine visual pigments by adenosine triphosphate. J. biol. Chem. **248,** 596–627 (1973)

Hagins, W. A.: The visual process; excitatory mechanisms in the primary receptor cells. Ann. Rev. Biophys. Bioengrg. **1,** 131–158 (1972)

Hagins, W. A., Penn, R. D., Yoshikami, S.: Dark current and photocurrent in retinal rods. Biophys. J. **10,** 380–402 (1970)

Hagins, W. A., Yoshikami, S.: A role for Ca in excitation of retinal rods and cones. Exp. Eye Res. **18,** 299–306 (1974)

Hagins, W. A., Yoshikami, S.: Ionic mechanisms in excitation of photoreceptors. Ann. N.Y. Acad. Sci. **264,** 314–325 (1975)

Hemminki, K.: Light-induced decrease in Ca binding to isolated bovine photoreceptors. Vision Res. **15,** 69–72 (1975)

Hendricks, T., Daemen, F. J. M., Bonting, S. L.: Light induced calcium movements in isolated frog rod outer segments. Biochim. biophys. Acta (Amst.) **345,** 468–473

Korenbrot, J. I., Cone, R. A.: Dark ionic flux and the effects of light on isolated rod outer segments. J. gen. Physiol. **60,** 20–45 (1972)

Liebman, P. A.: Light-dependent Ca content of rod outer segment disc membranes. Invest. Ophthal. **13,** 700–701 (1974)

Mason, W. T., Fager, R. S. Abrahamson, E. W.: Ion fluxes in disk membranes of retinal rod outer segments. Nature (Lond.) **247,** 562–563 (1974)

Montal, M., Korenbrot, J. I.: Rhodopsin in cell membranes and the process of phototransduction. In: The Enzymes of Biological Membranes (A. Martonosi, ed.). New York: Plenum Press 1976

Ostwald, T. J., Heller, J.: Properties of a magnesium or calcium dependent adenosine-triphosphatase from frog rod photoreceptors outer segments disc and its inhibition by illumination. Biochemistry **11,** 4679–4693 (1972)

Szuts, E. Z., Cone, R. A.: Rhodopsin: Light-activated release of calcium. Abstr. Biophys. Soc. **1974,** 1471 (1974)

Yoshikami, S., Hagins, W. A.: Control of the dark current in vertebrate rods and cones. In: Biochemistry and Physiology of Visual Pigments (H. Langer, ed.). Berlin-Heidelberg-New York: Springer 1973

Wormington, C. M., Cone, R. A.: Ca and H dependence, and ionic selectivity of the light-regulated Na channel in rod outer-segments. Abstr. Biophys. Soc. **1975,** 171a (1975)

Zimmerman, W. F., Daemen, F. J. M., Bonting, S. L.: Distribution of enzyme activities in subcellular fractions of bovine retina. J. biol. Chem. **251,** 4700–4705 (1976)

Received November 4, 1976

Discussion

P. Hillman, Jerusalem, Israel

I refer to your suggestion that, if your analysis of the conductivity of the rod disc is correct, the potential resulting from the transfer of a single change (proton) is sufficient to "pump" a few hundred Ca^{++} ions out of a disc in a few hundred milliseconds. I do not understand the significance of the calculation since, as soon as a single Ca^{++} ion has been pumped out by the potential change, this potential change has been reversed by the passage of that ion.