New and Notable

Rhodopsin Early Receptor Potential Revisited

Thomas P. Sakmar
Department of Molecular Biology and Biochemistry, Howard Hughes Medical Institute, Rockefeller University, New York, New York 10021 USA

In 1964, K. T. Brown and M. Murakami reported a flash-induced electrical response with no detectable latency in the retina. This early receptor potential (ERP) was characterized in some detail over the next several years by R. A. Cone, W. L. Pak, T. G. Ebrey and others in a variety of systems. The ERP is a biphasic response comprising an initial cornea-positive fast phase (R_1) followed by a slower cornea-negative (R_2) phase. The action spectra of both R_1 and R_2 matched that of rhodopsin, and the amplitudes of both phases were linearly proportional to the fraction of rhodopsin bleached by the stimulus flash. It was therefore proposed that the net displacement of electric charge in rhodopsin molecules undergoing light-dependent conformational changes generated the ERP. Since the charge displacement was proportional to the number of pigment molecules that photoisomerized, the ERP was a convenient method to obtain the photosensitivities of rods and cones. A. L. Hodgkin and P. M. O'Bryan (1977) used the ERP to obtain an apparent photosensitivity of turtle cone cells, and more recently C. L. Makino, W. R. Taylor, and D. A. Baylor (1991) determined photosensitivites by measuring the early receptor current (ERC) in voltage-clamped rod and cone photoreceptors from larval salamanders. The ERC is the charge motion underlying the ERP. They calculated the magnitude of the outward component (R_2) to correspond to the movement of about 0.18 *e* across the membrane per photoisomerization.

In an ambitious study in this issue of *Biophysical Journal*, J. M. Sullivan and P. Shukla describe the measurement of the ERC generated by rhodopsin heterologously expressed in tissue culture. They use a whole-cell gigohm-seal voltage clamp with millisecond-order time resolution to demonstrate that ERCs can be obtained from single or fused giant HEK293S cells expressing human opsin regenerated with 11-*cis*-retinal. The plasma membranes of these cells apparently contain rhodopsin in densities similar to that found in the plasma membranes of rod photoreceptor cells. The data show conclusively that the flash-activated ERC results from photoactivation of rhodopsin. This work has the potential to open a new approach to the study of the important problem of vectorial information transfer across cell membranes.

The ERC signal arises from charge displacement orthogonal to the plane of the membrane bilayer and is a probe of electric changes directed across the membrane dielectric. The ERC is kinetically complex, suggesting transitions among several contributing electrical states. The protein conformation changes underlying these transitions are unknown. However, the electrical difference between any two conformational states could be due to the net displacement of a bound charge, dipole reorientation, an induced structural dipole moment, or an interfacial charge migration such as proton uptake or release (Honig et al., 1986). Rhodopsin might well display all of these mechanisms as it undergoes a well-documented series of conformational changes initiated by the absorption of a photon of visible light.

Rhodopsin is an excellent model system for biophysical studies of the superfamily of G protein-coupled receptors (Sakmar, 1998; Gether and Kobilka, 1998). Molecular biological approaches in combination with various spectroscopic and biophysical methods have allowed the study of recombinant mutant pigments to address the physical basis of spectral tuning and the molecular mechanism of receptor activation (Kochendoerfer et al., 1999; Farrens et al., 1996). It has been known since the pioneering work of G. Wald and T. Yoshizawa that rhodopsin contains an 11-*cis* retinylidene chromophore, which isomerizes to the all-*trans* conformation upon photon absorption. But how is chromophore isomerization coupled to receptor activation? How do the conformational changes in the chromophore-binding pocket of rhodopsin propagate to the cytoplasmic surface of the receptor? What are the potential electrostatic changes associated with these conformational changes? The dominant interaction between chromophore and opsin is the electrostatic interaction between the retinylidene protonated Schiff base and its protein counterion, a glutamic acid residue. However, it is likely that both steric and electrostatic factors contribute to receptor activation (Shieh et al., 1997).

The primary photoproduct of rhodopsin, bathorhodopsin, relaxes through a series of spectrally defined intermediates, including lumirhodopsin, Meta I, and Meta II. There is good evidence that a protein conformational change occurs in the rhodopsin to Meta I transition, and that steric rather than electrostatic interactions are responsible for the structural changes leading to Meta I. Electrostatic interactions are more clearly involved in the subsequent conversion of Meta I to the active receptor, R*. Transfer of the Schiff base proton in the formation of Meta II is a key electrostatic trigger that precedes receptor activation. At least five concerted events are known to occur in forming the active R* conformation: retinal isomerization; Schiff base deprotonation; protonation of the

Received for publication 14 July 1999 and in final form 22 July 1999.

Address reprint requests to Thomas P. Sakmar, Dept. of Molecular Biology and Biochemistry, Rockefeller University, Howard Hughes Medical Institute, Box 284, RRB 515, 1230 York Ave., New York, NY 10021.

0006-3495/99/09/1189/02 $2.00

Schiff base counterion; transmembrane helix movement, primarily of helical segments 3 and 6; and proton uptake at the cytoplasmic surface of the receptor. Schiff base deprotonation and subsequent protein conformational changes are likely to be driven by electrostatic interactions. These conformational changes should all contribute to the ERC waveform. In particular, it is likely that the R_2 phase of the ERC represents in part the conversion of Meta I to Meta II.

The mechanistic details of electrically active conformational changes in membrane proteins are of great interest. Since it is not unusual for biological membranes to have significant transmembrane potentials, membrane proteins may be exposed to intense electric fields. Their structural conformations may be functionally coupled to the electric field for the purpose of switching, transport, or energy transduction (Honig et al., 1986). For example, ion channels undergo conformational changes that open and close an ion-permeable pore across the membrane. In voltage-dependent channels this gating process is influenced by transmembrane voltage. Charge motions in rhodopsin that result in the ERC are conformation-dependent currents, as are ion channel gating currents. Perhaps not surprisingly, the ERC is remarkably similar in waveform and kinetics to an ion channel gating current. In voltage-dependent ion channels, conformational states cannot be measured independently of gating current. However, in rhodopsin a flash of light can change the conformational state, and the conformational changes can be probed independently of the ERC by a variety of spectroscopic techniques including time-resolved absorption spectroscopy. Several investigators including C. F. Stevens and R. Ranganathan have previously noted that the ERC is akin to a rhodopsin gating current. J. M. Sullivan and P. Shukla also address this notion.

The work by J. M. Sullivan and P. Shukla provides an exciting new method to study the relationship between structure and electrostatics in membrane protein receptors. It is reasonable to expect that this method will bring a new dimension to structure-function studies of rhodopsin and other visual pigments. In particular, it should be possible to correlate specific features of the ERC to specific electrostatically based conformational changes in rhodopsin. It is reasonable to expect that site-specific mutants of rhodopsin that are expressed at high levels in the plasma membrane will be amenable to study. This approach would allow the identification of the molecular components responsible for R_2. A major challenge will be to design experiments to identify the mechanistic events that underlie the ERC. These could include chromophore dipole reorientation, charge separation, dipole movement, intramolecular proton transfer, and vectorial proton uptake or release at the aqueous interface.

REFERENCES

Brown, K. T., and M. Murakami. 1964. A new receptor potential of the monkey retina with no detectable latency. *Nature*. 201:626–628.

Farrens, D. L., C. Altenbach, K. Yang, W. L. Hubbell, and H. G. Khorana. 1996. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science*. 274:768–770.

Gether, U., and B. K. Kobilka. 1998. G protein-coupled receptors. II. Mechanism of agonist activation. *J. Biol. Chem.* 273:17979–17982.

Hodgkin, A. L., and P. M. O'Bryan. 1977. Internal recording of the early receptor potential in turtle cones. *J. Physiol.* 267:737–766.

Honig, B. H., W. L. Hubbell, and R. F. Flewelling. 1986. Electrostatic interactions in membranes and proteins. *Annu. Rev. Biophys. Biophys. Chem.* 15:163–193.

Kochendoerfer, G. G., S. W. Lin, T. P. Sakmar, and R. A. Mathies. 1999. How color visual pigments are tuned. *Trends Biochem. Sci.* 24: 300–305

Makino, C. L., W. R. Taylor, and D. A. Baylor. 1991. Rapid charge movements and photosensitivity of visual pigments in salamander rods and cones. *J. Physiol.* 442:761–780.

Sakmar, T. P. 1998. Rhodopsin: a prototypical G protein-coupled receptor. *Prog. Nucleic Acid Res. Mol. Biol.* 59:1–34.

Shieh, T., M. Han, T. P. Sakmar, and S. O. Smith. 1997. The steric trigger in rhodopsin activation. *J. Mol. Biol.* 269:373–384.

Sullivan, J. M., and P. Shukla. 1999. Time-resolved rhodopsin activation currents in a unicellular expression system. *Biophys. J.* 77: 1333–1357.