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New and Notable

Exquisite Sensitivity of Electroreceptor in Skates

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Sensory receptors in organisms are known to exhibit remarkable sensitivity. A few molecules of chemical attractant, such as pheromones or odorants, are sufficient to influence the behavior of moths and ants, and a few photons are needed to produce a visual sensation (Nicholls et al., 1992). This type of sensory reception mostly involves initial activation of ion channels by a substance or stimulus for the electric amplification of signal, followed by a cascade of enzymic reactions that bring about the biological responses. Although detailed mechanisms of transduction could be complex, the phenomenon is less a puzzlement to scientists than another type of sensory reception, the reception of signals that have very low energy content compared with the activation energy for most biochemical processes. Examples for the latter include the response to electric signals in nV/cm by electric fishes (Kalmjin, 1982; Heiligenberg, 1989) and to auditory signals that produce mechanical vibration of hair cells of the order of nm (Hudspeth, 1989). The crucial question is, how can such a weak stimulus, either electrical, magnetic, acoustic, or mechanical, activate the biochemical responses? The Ampulla of Lorenzini of skates is a good organ to study for this sensitivity and for mechanisms for achieving such sensitivity.

In a paper appearing in this issue, Lu and Fishman (1994) examine the electric sensory responses of the apical and basal membrane ion channels of the innervated ampullary organ, excised from skates. They have found that an

intact organ can exist in two different operational states, one with spontaneous repetitive voltage spikes (State I) and the other with evoked spikes (State II), both of which are reflected in the spike firing rate of the afferent nerve. Admittance and step voltage clamp measurements and analyses of the isolated organs have shown that a negative conductance underlies the ampullary epithelial spiking in States I and II. Further, Lu and Fishman found that changes in the spike firing rate of the afferent nerve were detectable for voltage clamps of the ampullary epithelium of only a few μV and saturated at 100 μV . These results indicate that isolated ampullary organs can respond to electric field levels that are comparable with those observed previously in intact animals. Their results also suggest that the high sensitivity of electroreception involves a negative epithelial conductance (current flow in direction opposite to the membrane depolarization) that can amplify the transorgan electric signal according to the Eq. 6.

The sensitivity shown in these *in vitro* experiments is close to, but not yet reaching, the level that has been reported in the behavior studies (Kalmjin, 1982; Heiligenberg, 1989). What limits the ability of an organism to sense an electric or an electromagnetic signal? One assumption is that sensory transduction is limited by thermal noise. A signal with an energy level below the thermal noise of the cell membrane would be masked by these noises and would not be perceptible by cells or organisms (Weaver and Astumian, 1990; Adair, 1991). Is an electric field in the range of nV/cm or $\mu\text{V}/\text{cm}$ sufficiently strong to rise above the thermal electric noise? Can membrane amplification, or time averaging of, or coherent response of an ensemble of molecules to a signal enhance the sensitivity of a sensory organ (Weaver and Astumian, 1992)? Others consider thermal fluctuation irrelevant to, or a source of rather than a limitation to, the ability of an organism to sense

signals of very low energy level (Maddox, 1994; Douglass et al., 1993; Markin et al., 1992). And what could be the mechanisms by which molecules recognize, respond, and process periodic signals (Tsong and Astumian, 1986; Tsong, 1992)? These are relevant questions to ask for resolving the molecular basis and mechanisms of the exquisite sensitivity of sensory transduction in cells and organisms. Does it take a sumo san to move a 10-ton pendulum? The answer is no. Anyone who knows how to use a periodic forcing which matches the period of the pendulum will be able to overcome its inertia. A periodic forcing, or an RTF (random telegraph fluctuation) forcing, is like a signal, and it is unlikely to be masked by an erratic forcing (noise) even if the signal is much weaker than the noise.

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Analyzing the Structure of Polypeptides in Membranes by Fluorescence Quenching

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One of the most difficult problems in membrane biophysics is the determination of the location of molecules within the membrane. For membrane peptides and proteins the problem is especially severe, as formidable barriers remain to the crystallographic and nuclear magnetic resonance techniques that are so useful in solution. Therefore, the development of alternate methods is of great interest. It is becoming increasingly attractive to use fluorescence to locate groups within membranes. Such a strategy requires a reliable method to determine the depth of a fluorescent group within the membrane. This has proven to be much harder than expected. Among the more powerful methods is the use of membrane-bound fluorescence quenchers such as brominated or nitroxide-labeled lipids. Nitroxide-labeled lipids are coming into wide use for such applications because they have the ability to quench a wide range of fluorophores, and their location in the membrane has been fairly well defined. In addition, a simple equation that allows the calculation of depth from the amount of quenching has been developed, and the validity of the resulting analysis has been tested using a wide variety of membrane-bound fluorophores. This "parallax analysis" approach involves the comparison of

the intensity of fluorescence in two different samples in which phospholipids that carry a quenching group at different, but defined, depths have been incorporated into the bilayer (Chattopadhyay and London, 1987; Abrams and London, 1993).

To solve the structure of a peptide or protein by fluorescence quenching a unique fluorescent site must be introduced in the form of a single Trp residue or single fluorescence-labeled Cys. By localizing the membrane location for a series of such mutants, the membrane penetration of an entire polypeptide chain could potentially be mapped out in detail. In this issue Jones and Gierasch (1994) begin to look at the insertion of a signal sequence peptide by combining introduction of a single Trp with fluorescence quenching by nitroxides. Other groups have recently applied this approach to both peptides and membrane proteins (Chung et al., 1992; Palmer and Merrill, 1994).

Jones and Gierasch (1994) start with control experiments using both Lys-Trp-Lys, and a peptide with a 20-residue-long poly-Ala-Val stretch in which a Trp has been incorporated near either the center or the ends. They demonstrate the basic reliability of the approach by obtaining the expected surface location for the Trp in the former case, and the expected nearly transmembranous conformation in the latter.

The main experiments involve the LamB signal sequence peptide with a Trp fixed at either toward the middle (W18) or C-terminal (W24) of the peptide. Quenching detects a deep insertion of these peptides. Jones and Gierasch (1994) interpret these results in terms of a model in which an equilibrium exists between a predominant nontransmembranous but membrane-penetrating helix that is tilted from the membrane surface and a less favorable transmembranous structure. Introduction of ionizable residues toward the center of the peptide tend to decrease the overall depth of penetration, perhaps decreasing the degree of penetration by decreasing the tilt in the nontransmembranous form.

One important observation made by Jones and Gierasch (1994) is that the wavelength of maximum emission is correlated with depth much more weakly than would be hoped. The well known observation that fluorescent groups emit at progressively shorter (blue-shifted) wavelengths as polarity is decreased could potentially be used to measure the depth of a residue in the membrane. It might be hoped that the deeper a residue is located in the hydrophobic core of the membrane, the more blue-shifted it would fluoresce. However, Jones and Gierasch (1994) find introduction of an Asp residue within the most hydrophobic regions of the LamB signal peptide largely abolishes the blue shift of Trp in the 18 position, despite the fact that nitroxide quenching shows the Trp remains deeply embedded in the membrane. Furthermore, the introduction of an Arg residue has only a weak effect on the emission wavelength, despite resulting in a perceptibly shallower Trp depth. The authors mention several possible explanations for the surprisingly reduced blue shift in the Asp peptide, favoring increased hydration near the Trp residue due to the presence of Asp. Intriguingly, low pH reestablishes the expected blue shifts of Trp fluorescence in these peptides. Whatever the explanation, the dangers of using an indirect method such as maximum emission wavelength to estimate depth are clearly highlighted by these results.

As might be expected, the observations of Jones and Gierasch (1994) raise almost as many questions about the structure of the LamB polypeptide as they answer. Is the weak quenching of the Asp peptides related to some phenomenon, such as peptide aggregation within the membrane, that could also explain the lack of a Trp blue shift? What is happening to the structure of the Asp containing peptides at low pH where the blue shift returns to expected values? Does the introduction of Trp affect peptide structure? Furthermore, Jones and Gierasch (1994) correctly point out that the absolute values of the depths obtained from the parallax analysis are subject to uncertainties at this stage of our understanding. Are