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Visual Transduction: Chairman's Introduction

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The functioning of the visual apparatus has attracted the curiosity of scientists for centuries, but is is only the past fifteen years that the molecular mechanisms of transduction from light to voltage transients have become accessible to experimental investigation. The dynamic properties of the vertebrate retina are exceptional. Extreme sensitivity in the low-light domain is joined with very high adaptability to high light intensity. One quantum of light can electrically excite a single rod cell [Yau et al, 1977; Baylor et al, 1979], and the whole retina is adaptable to light intensities that vary over 12 orders of magnitude

Figure 1 is a simplified representation of a rod cell in a vertebrate retina. The outer segment of this cell carries a system of some 1,000 disks, which incorporate 97% of the light-sensitive pigment, rhodopsin, in their membrane. The inner segment contains the nucleus and mitochondria. By synaptic contact it is linked to a network of nerve cells, which is finally connected to the visual cortex of the brain. A Na^+/K^+ pump operates in the plasma membrane of the inner segment. It is believed that K^+ is short-circuited in the inner segment. Na^+ flows along the rod to the outer segment where it traverses the plasma membrane through light modulated Na^+ channels. The sodium current polarizes the plasma membrane in the dark. Excitation of rhodopsin by light causes hyperpolarization of the cell. This could be attributed to closure of Na^+ channels [Bortoff and Norton, 1967; Baylor and Fuortes, 1970; Penn and Hagins, 1972; Yau et al, 1981]. Since it is generally believed that disk membranes are morphologically and electrically separated from the plasma membrane [Penn and Hagins, 1972], it has been inferred that information transfer from rhodopsin in disks to Na^+ channels in the plasma membrane is mediated by a transmitter substance. The search for the chemical nature of the transmitter and for the mechanism of transmission has dominated this field for the past decade.

GENERAL FEATURES OF SIGNAL TRANSMISSION

Signal-to-noise considerations have led to the conclusion that single quantum causes the closure of more than 100 Na^+ channels in the plasma mem-

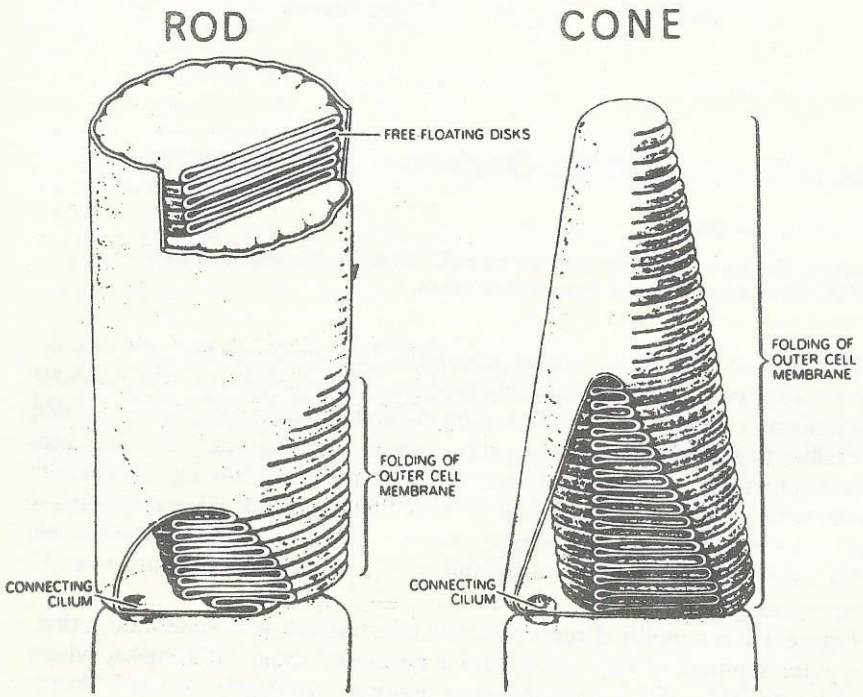

Fig. 1 Schematic representation of a rod cell in a vertebrate retina [Young, 1970].

brane [Yoshikami and Hagins, 1973]. The shape of the electric response to a flash of light, and in particular the length of its delay, depends on the light energy. However, when a rod was excited with one photon, the delay did not change from one trial to the next [Baylor et al, 1979b]. This suggested that the velocity of the electric response was not limited by radial diffusion of transmitter molecules in or at disk membranes. On the other hand, there was only limited spread of excitation along the axis of a rod (3 μm) after illumination of a narrow slice of the cell [McNaughton et al, 1980]. This is suggestive of diffusion control along the long axis of a rod. The delay and the rise of the electric response was kinetically complex, and it could be fitted by at least four consecutive reactions between light absorption and channel closure [Baylor et al, 1979a].

THE Ca^{2+} TRANSMITTER HYPOTHESIS

In 1971 Yoshikami and Hagins proposed a unified concept for visual transduction, amplification and transmission [see also Yoshikami and Hagins,

1973]. They postulated that disks were pumped full of Ca^{2+} in the dark. When rhodopsin was hit by a quantum of light, a channel was formed that unloaded the clacium pool into the cytoplasm. The increase of the cytoplasmatic Ca^{2+} concentration caused the closure of Na^{+}-channels in the plasma membrane. It was most attractive that in this hypothesis only one species, namely, Ca^{2+}, was required to explain both aspects of visual transduction, amplification and transmission. However, the postulated exclusive role of calcium was impossible to prove. The hypothesis was based on the observation that an increase of the calcium concentration in the bathing solution of a retina mimicked the effect of illumination on the electric behaviour of rod cells [Yoshikami and Hagins, 1971,1973]. This was confirmed in other laboratories [Brown and Pinto, 1974; Lipton et al, 1977; Bastian and Fain, 1979]. The action of extracellular calcium on rods, however, was not directly related to the calcium transmitter hypothesis. Therefore it was attempted to modify the intracellular concentration of calcium and to monitor the effects on the sodium channels in the plasma membrane. These experiments, however, were difficult and their interpretation even more so. In particular, it was not possible to assess whether the injection of Ca^{2+} was able to create a sufficiently large concentration change in competition with the cytoplasmic buffering capacity and possible calcium transport systems [for detailed arguments see B. Kaupp, this volume].The two important elements of the Ca^{2+}-transmitter hypothesis are a Ca^{2+}-pump and a light-modulated Ca^{2+} permeability, both in the disk membrane. The search for a calcium pump which would drain on the cellular pool of high-energy phosphates in the presence of Ca^{2+} ionophores gave negative results [Berman et al, 1977; Schnetkamp, 1981]. The search for light-stimulated release of calcium from disks gave conflicting results [see U.B. Kaupp, this volume]. It was assumed that the mechanism of visual transduction would be conserved among rods and cones. Cones resemble rods with disks that are contiguous invaginations of the plasma membrane. Addition of Ca^{2+} to cones failed to inhibit the photoresponse [Arden and Low, 1978; Bertrand et al, 1978]. This was considered as indirect evidence against the calcium-transmitter hypothesis. In photoreceptors of certain invertebrates the calcium concentration changed transiently in response to light, however, this took part in adaptation rather than in signal transmission [Brown and Blinks, 1974; see H. Stieve, this volume].

In conclusion, the Ca^{2+}-transmitter hypothesis could not be verified until now. Rather its credit has declined. Some recent observations, however, indicate that Ca^{2+} is all but out of the game. Studies with whole retinae showed a huge calcium extrusion after illumination. The amplification was greater than 10^3 calcium per bleached rhodopsin [Yoshikami et al, 1980] and

5×10^4 [Gold and Korenbrodt, 1980]. Very recently, George and Hagins [1983] reported that they could overcome the so-far-observed reluctance of disks to store and to release sufficient quantities of calcium by bathing disks in appropriate media, which among others contained cyclic GMP.

ON THE ROLE OF CYCLIC GMP

Light stimulates hydrolysis of cyclic GMP (cGMP) in retinal rods. This had led to the suggestion that cGMP could act as the transmitter substance [Miki et al, 1975; Liebmann and Pugh, 1979]. The amplification was high; up to 4×10^5 cGMP were hydrolyzed per photoexcited rhodopsin [Liebmann and Pugh, 1979]. However, it was clear from the beginning that the resulting average changes of cGMP concentration in a rod cell were rather small (some 10%). Activation of the cGMP phosphodiesterase is the result of a multi step process: first one photoexcited rhodopsin undergoes several transitions until meta-rhodopsin II is formed; this then stimulates some 500 molecules of a GTP-binding protein. Each of these in turn activates one molecule of a cyclic-GMP-phosphodiesterase (PDE). One diesterase hydrolyzes in the order of 1,000 molecules of cGMP during its activity period [Kühn, 1980; Fung and Stryer, 1980; Fung et al, 1981]. The mechanism of this activation chain has been elucidated in great detail [see H. Kühn, this volume].

Attempts to demonstrate a direct effect of cGMP on the Na^+-channels in the plasma membrane yielded conflicting results. There was one report which suggested such direct action [Nicol and Miller, 1978]. Intracellular injection of cGMP caused depolarization of the plasma membrane and an increased delay of the photoresponse (negative transmitter). Other authors found no straightforward correlation between the intracellular level of cGMP and the response of rods to light [Woodruff and Fain, 1982]. Perhaps the most convincing evidence against a direct transmitter role of cGMP came from kinetic studies wherein the velocity cGMP hydrolysis was correlated with the rise of the electric response. Two groups used rapid-freezing techniques [Kilbride and Ebrey, 1979; Govardowskii and Berman, 1981] and one followed the internal pH-changes, which they considered as indicative of cGMP hydrolysis via pH-indicating dye [Parkes and Liebman, unpublished]. Under excitation with light pulses of low energy the onset of cGMP hydrolysis showed a longer delay (3 sec) than the onset of the transient hyperpolarization [see T. Ebrey, this volume].

SYNTHESIS

We are left with an exciting situation. Several elements of the causality chain of visual transduction have been spotted, but the most important link is

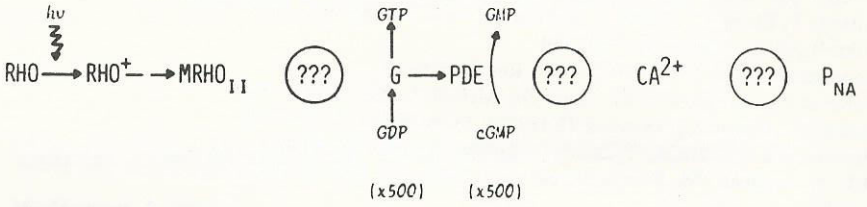

Fig. 2 Elements of visual transduction in vertebrate rods. RHO, rhodopsin; $MRHO_{II}$, metarhodopsin II.

missing. The components are illustrated in Figure 2: rhodopsin, the enzyme cascade that finally hydrolyses cGMP, calcium cations, and the sodium channels in the plasma membrane. cGMP is possibly involved in the chemical amplification of the light stimulus, while it is probably not the transmitter per se. Calcium ions can act on the sodium channels, and they may be the transmitter, but their exclusive role both in amplification and transmission could not be verified.

The pathways for calcium and for cGMP are intimately connected [Bownds, 1981]. The mechanistic link, however, is missing. It appears as if the effector is not the concentration of cGMP proper, but some product of rapid cGMP hydrolysis. It has been proposed that protons that are liberated during cGMP dydrolysis expel calcium ions from binding sites, which in turn act on the sodium channels in the plasma membrane [Mueller and Pugh, 1983]. This however, remains to be established.

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