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Visual transduction in vertebrate rods

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Recent studies of visual transduction in vertebrate rods, the photoreceptors for dim light, at physiological and molecular levels are reviewed.

Key terms: *dark noise, light adaptation, rhodopsin evolution, spectral sensitivity, sensory physiology, vertebrate visual transduction*

REVIEW

Introduction

Understanding visual transduction in photoreceptors has been a major focus of research during the last couple of decades. Following is a very brief overview of some of the major findings on rods –the retinal photoreceptors that function in dim light. For more extensive recent reviews see Baylor (1996), Koutalos and Yau (1996) and Lamb (1996).

Visual transduction

Rhodopsin, the pigment molecule of rods that absorbs light for scotopic vision, is an integral membrane protein (40 kDa) that makes seven helical traverses of the membrane. It is covalently bonded to 11-*cis* retinal through a Schiff's base linkage with the ε-amino group of a lysine residue (Lys296) (Nathans, 1990; Yau, 1994). In complete darkness the resting potential (~-40mV) of rods reflects the presence of an inward current of Na^+ (80%) and Ca^{++} (15%) through guanosine 3',5'-cyclic monophosphate (cGMP) gated channels in the outer-segment membrane (Kaupp, 1995) and extrusion of K^+ at the inner segment. Photoisomerization of retinal from the 11-*cis* to the all-*trans* configuration induces conformational changes in the protein which in turn lead to the activation of a G-protein which in turn removes the inhibition on phosphodiesterase (PDE*). PDE* hydrolyzes cytoplasmatic cGMP, causing the Na^+ channels to close and the membrane to hyperpolarize (Yau and Baylor, 1989). Lamb (1996) has recently published a stochastic model of photoactivation through this G-protein cascade.

Kinetics of the rod response

The reduction in the flow of ions –the photocurrent– has been measured by drawing rod outer segments into pipette-shaped electrodes (Baylor, 1996). We describe here some of these results.

The time course and the amplitude of a rod response to dim flashes is linearly related to photon flux and can be described by a series of slow filter stages (n = 4-6) (Baylor *et al*, 1979). At higher intensities, rod responses reach saturation (Fig 1A). When plotted as a function of log intensity, the data can be fit to a simple exponential function of the form $1\text{-}e^{-kI}$ where I is the intensity of the flash (Lamb and Pugh, 1992) (Fig 1B). The kinetics of the rising phase of the response reflects biochemical activation processes and can be understood in terms of the G-protein

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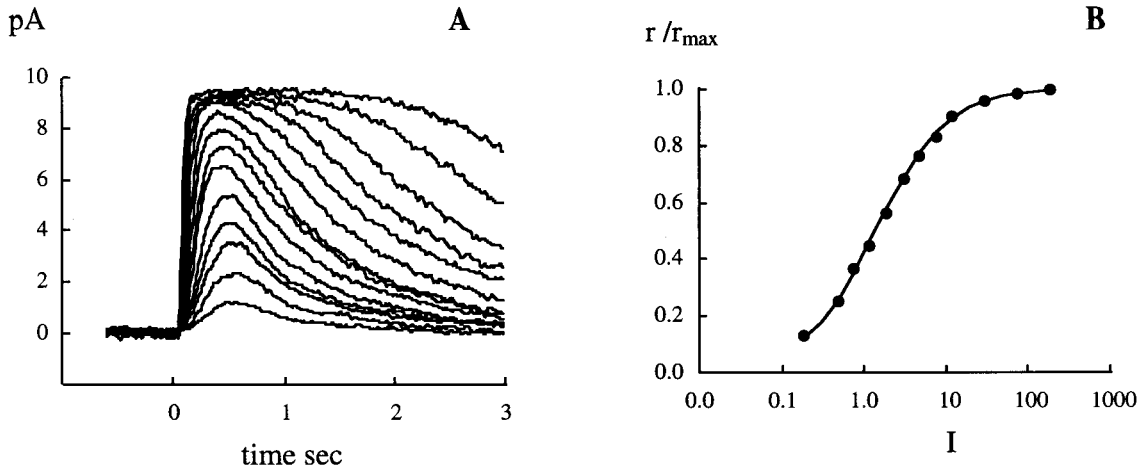

Fig 1. **A**. Family of responses of an isolated *Rana pipiens* rod to 10 ms flashes of 500 nm of polarized light of increasing intensity. The dimmest flash was 0.19 photons μm^{-2}. The lower trace is an average of twelve responses. **B**. Normalized response-energy function. The continuous curve is the best fit to a simple exponential function. For method and data analysis, see Palacios and Goldsmith (1993).

Table I

Parameters characterizing rods responses to dim flashes

Class / Species	S_ϕ	I_0	S_ϕ/r_{max} x 100	t_{peak} ms	t_1 ms	T°C	Reference
Fish							
Skate	0.48	36	4.0	800	–	20	Cornwall *et al*, 1989
Bass	0.73	22	2.0	342	–	20	Miller *et al*, 1993
Amphibian							
Bufo	0.34	61	2.0	1290	–	22	Baylor *et al*, 1979
Newt	0.28	107	0.9	950	–	20	Forti *et al*, 1989
Salamander	0.29	59	1.7	810	1180	20	Palacios *et al*, 1996
Rana	0.21	49	2.1	660	1240	20	Palacios *et al*, 1996
Reptile							
Gecko	0.78	50	1.2	1100	–	18	Rispoli *et al*, 1991
Bird							
Pigeon	0.37	83	1.2	327	851	39	Palacios *et al*, 1993
Mammal							
Human	0.10	66	0.9	189	316	37	Kraft *et al*, 1993
Cynomolgus monkey	0.49	33	2.1	189	291	36	Baylor *et al*, 1984
Rhesus monkey	0.97	19	5.4	233	313	38	Tamura *et al*, 1991
Green monkey	0.77	17	5.9	211	334	38	Tamura *et al*, 1991
Bush baby	0.59	17	5.9	203	298	38	Tamura *et al*, 1991
Cattle	0.57	23	4.4	219	295	39	Nakatami *et al*, 1991
Rat	0.54	11	9.0	238	333	39	Nakatami *et al*, 1991
Rabbit	0.81	16	6.2	161	376	39	Nakatami *et al*, 1991
Cat	1.12	12	8.6	154	263	39	Nakatami *et al*, 1991

cascade provided that cytoplasmatic Ca^{++} is chelated (Lamb, 1996). The kinetics of the deactivation process is less well understood, although, serine and threonine residues in the carboxyl terminal of the opsin terminal are phosphorylated and Ca^{++} ions are known to participate (Baylor, 1996).

Table I summarizes some of the quantitative parameters that characterize the responses of rods to dim flashes: S_ϕ is the amplitude response in pA to single activated rhodopsins (*Rh**), I_0 is the intensity of light required for a half-maximal photocurrent, measured here as the number of activated rhodopsins, $Rh^* \cdot S_\phi / r_{max}$ is the fraction of the total possible photocurrent suppressed by a single isomerization, t_{peak} is the time-to-peak and t_I is the integration time (Baylor *et al*, 1979). After adjusting for a Q_{10} of 2.7 (Baylor *et al*, 1983), the kinetics of vertebrate rod responses from different species are comparable, except that the rods of pigeon (*Columba*) generate responses to single photons with smaller amplitudes, slower time-to-peak, and longer integration times than mammalian rods (Palacios and Goldsmith, 1993).

Dark noise

Spontaneous fluctuations in the membrane conductance –an electrical dark noise– impose a threshold barrier that potentially limits the sensitivity of rods. Dark noise (other than instrumental noise) consists of two contributions, a more frequent component of small amplitude (average 0.2 pA in toad, 0.029 pA in monkey) which has been attributed to random fluctuations in molecular components of the transduction cascade, and discrete events, similar in shape and amplitude (1 pA in toad) to single photoisomerizations (Yau and Baylor, 1989; Baylor, 1996). The latter vary in their rate of occurrence: 0.02-0.03 s^{-1} in toad (20°C; Baylor *et al*, 1980), 0.005 s^{-1} in bull-frog (18°C; Donner *et al*, 1990) and 0.006 s^{-1} in monkeys (36°C; Baylor *et al*, 1984). Interspecific comparisons suggest that the frequency of occurrence of discrete events increases in direct proportion to the content of rhodopsin (Birge and Barlow, 1995).

In toads, the dependence of the rate of occurrence of discrete events with temperature corresponds to an activation energy of 22 kcal mol^{-1}. The origin of discrete events has frequently been attributed to thermal isomerization of rhodopsins molecules (Baylor *et al*, 1980; but see Goldsmith, 1990; Barlow *et al*, 1993).

Single conductance

The conductance of single, cGMP-gated, light sensitive channels that are permeable to a variety of divalent cations have been studied in most detail in amphibians. In the absence of divalent cations, the amplitude of response ranges from 20-25 pS measured with a patch clamp, to 55-60 pS measured by statistical analyses of discrete events. Under physiological conditions, however, the unit conductance is much lower, ~0.1 pS (Yau and Baylor, 1989).

Light adaptation

Background light causes a reduction in gain (pA/photon) and a decrease in time-to-peak of response. The reduction in gain increases the dynamic range of the cell by increasing the light required for saturation of the response. The mechanism controlling light adaptation involves a lowered internal concentration of Ca^{++} as channels close in the light and Ca^{++} continues to be pumped from the cell. Lowered internal Ca^{++} removes inhibition of guanylate cyclase (the enzyme that forms cGMP from GTP) and decreases the activation of the transduction cascade (Koutalos and Yau, 1996; Mathews, 1996).

Spectral sensitivity

The spectral sensitivity of rods is determined by the probability of photon capture and is a function of wavelength. Rhodopsin has three absorption bands: the α-band which depends on the protonated Schiff-base linkage between retinal and the protein (λ_{max} at about 500 nm), the ß-band which probably reflects the presence of the *cis* isomer of retinal (λ_{max} at about 340 nm), and the γ-band due to aromatic amino acids on the protein moiety

of the opsin (λ_{max} at about 280 nm; Collins *et al*, 1952). For light absorbed by the γ-band, the probability of isomerization is substantially less than at longer wavelengths. Moreover, there is virtually no natural, environmental light at such short wavelengths, so the γ-band contributes nothing to spectral sensitivity *in vivo*. It is nevertheless possible to show energy transfer from the γ-band by studying isolated rods (unpublished observations of the authors). For spectral sensitivity in vertebrate rods see: birds, pigeon (Palacios and Goldsmith, 1993); fish, bass (Miller and Korenbrot, 1992); mammals, human (Kraft *et al*, 1993), monkey (Baylor *et al*, 1984), squirrel (Kraft, 1988); amphibians, salamander and frog (Palacios *et al*, 1996), and toad (Baylor *et al*, 1979). For a discussion of the position and spectral dependence of λ_{max} on point mutations, see Yokoyama (1995).

Vertebrate rhodopsin

Rhodopsin belongs to a large family of G-protein-coupled receptors (Rens-Domiano and Hamm, 1995). In the last few years, the genes for 15 different vertebrate opsins have been reported (Smith *et al*, 1995). The phylogenetic distribution of rhodopsins shows highly conserved homology. Although rhodopsins seem to have arisen from the opsins that are now found in cones (Okano *et al*, 1995), the divergence occurred in deep evolutionary time, perhaps even before the presence of vertebrates in the fossil record (Harosi and Kleinschmidt, 1993; Goldsmith, 1994).

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