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On the Mechanism of Conductance Control of the Arthropod Visual Cell Membrane* **

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Abstract. It is assumed that "dark channels" determine a permanent dark conductance of the arthropod visual cell membrane. The light stimulus causes a transient opening of "light channels". The ion selectivity of dark channels and light channels is roughly described. Factors influencing the activation of light channels, as membrane energy metabolism, membrane potential and adjusted calcium ion concentration are specified. The mechanism of the action of calcium ions on the conductance of the visual cell membrane is discussed.

Key words: Visual cell membranes – Invertebrates – Ion channels – Activation of light channels – Calcium.

The membrane potential of the arthropod photoreceptor cell is mainly determined by ionic gradients and selective ion permeabilities of the membrane. It seems reasonable to assume that ion channels are responsible for the ion-specific conductance of the visual cell membrane. Under these premises one has to assume at least two types of ionic channels, "dark channels" and "light channels".

1. *Dark channels* are responsible for a permanent dark permeability. They are either permanently open, or their opening and closing fluctuates and a certain fraction of all dark channels is open at a given time; the opening of the dark channels is not activated by light.

There are indications that there is more than one type of dark channels. The strongest indication is the rudimentary electrical excitability of dark photoreceptors (which can only be explained by more than one type of channels).

The conductance of the photoreceptor cell membrane in the dark is in the order of $10^{-5}\ \Omega^{-1}\ cm^{-2}$ (Millecchia and Mauro, 1969; Stieve and Hartung, 1976).

If a dark channel has a conductance of $2–40 \times 10^{-12}\ \Omega^{-1}$ this corresponds to a density of $10^{-3}–10^{-1}$ open dark channels per μm^2, which amounts for the retinular cell of *Astacus* to 10^2 to 10^4 open dark channels per retinular cell (Stieve and Hartung, 1976).

* These considerations are mainly concerned with photoreceptor cells of *Limulus*, *Astacus* and *Balanus*

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Table 1. Relative ion specificity of dark channel

X	K^+	Rb^+	Na^+	Cl^-
P_X/P_K	1	0.7	0.04	0.3

Table 2. Relative ion specificity of light channel

X	Na^+	Li^+	K^+	$Cholin^+$	$Tris^+$	Ca^{++}
P_X/P_{Na}	1	1	ca. 0.5	< 0.5	< 0.2	> 0

Table 1 shows the relative ion specificity of the dark channel as referred to the permeability for potassium ions. It is assumed that only one type of dark channel determines the ion permeability of the membrane in the dark and that the ionic preference of the dark channel is not membrane potential dependent. Possibly dark channels have similarities to the leakage channels of the nerve membrane.

The values for Na^+ and Cl^- are from Brown (1976), derived from measurements with ion selective microelectrodes in *Balanus* photoreceptors, and the value for Rb^+ is from Stieve and Hartung (1977) by dark tracer efflux measurements in *Astacus* retinular cells.

2. *Light channels* are opened transiently following the light stimulus. For a saturating light stimulus the conductance of the visual cell membrane increases by a factor greater 10 – presumably even greater 100.

The total number of light channels of a retinular cell has not yet been measured and is probably very high. Kramer (1976) estimates a number which is greater than 10^5 per retinular cell. In the *Astacus* retinular cell it is probably much greater than 10^5, the number of microvilli and possibly even as great as the number of rhodopsin molecules (10^9–10^{10}).

The light channel can be described as having a preference for sodium ions over potassium ions and probably a certain permeability for calcium ions. Table 2 shows estimates of the relative ions specifity of the light channel as referred to the permeability for sodium.

The values of Li^+, K^+, $Cholin^+$ and $Tris^+$ are estimates of Brown and Mote (1974), measured by the voltage clamp reversal potential of the light induced membrane current of a *Limulus* ventral nerve photoreceptor.

Perhaps there exist two types of light channels. Besides the above characterized light channel which may be called "early" light channel, there may possibly exist a second type of light channel, which is activated in the course of the light response. This "late" light channel may prefer potassium ions. Indications for its existence come from the observation that the light induced conductance increase decays more slowly than the receptor potential.

Activation of Light Channels

The activation of the (early) light channels is not caused by a change in membrane potential but by a causal chain of events initiated by the absorption of a photon by a rhodopsin molecule. Photon absorption causes changes in the rhodopsin molecule which induce an amplification process. This in turn causes a greater number of light channels to be transiently opened (Fig. 1).

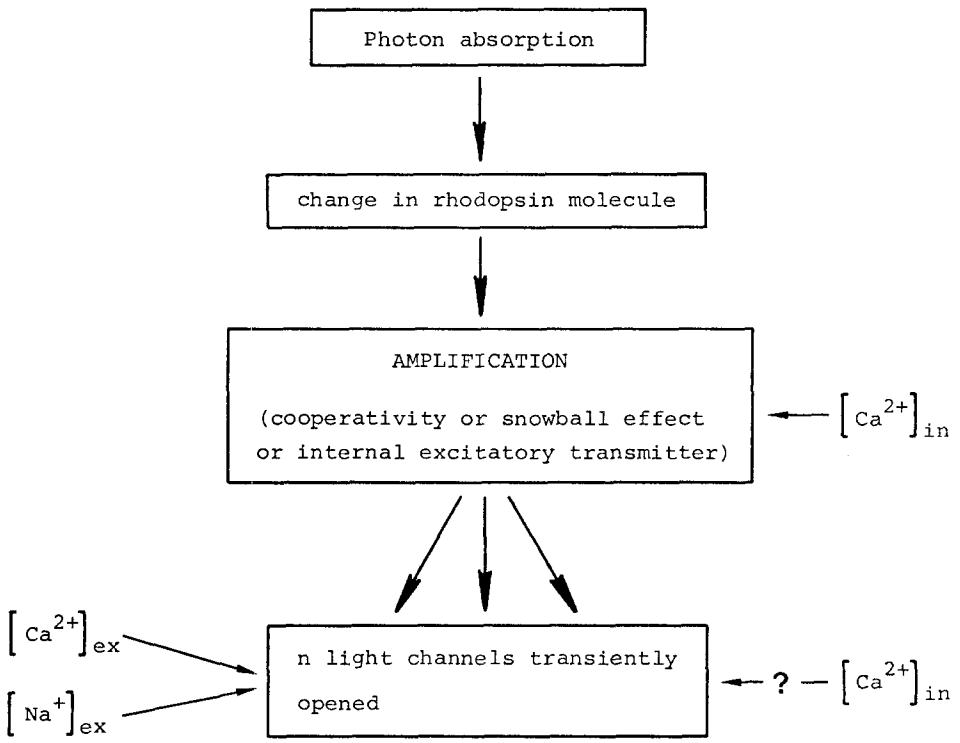

Fig. 1. Causal chain of visual excitation

The amplification could be brought about either by an internal excitatory transmitter (not calcium) or by a snowball effect reaction chain or – e.g. if the rhodopsin molecule itself is a light channel forming molecule – by cooperativity between rhodopsin molecules.

In the dark adapted photoreceptor this amplification is very high: Borsellino and Fuortes (1968) deduct, from model calculations for the *Limulus* retinular cell, a number of 25 molecules to be influenced by one rhodopsin molecule having absorbed a photon. Cone (1973) estimates that 100 light channels are opened due to a photon absorption by one rhodopsin molecule at threshold. Kramer (1975) assumes that under the same conditions 10^3–10^5 light channels are opened (if a light channel has a conductance of 10^{-11} Ω^{-1}).

The light channels can only be activated by light if certain prerequisite conditions are met:

1. The excitability of the light channels disappears when the energy metabolism of the cell membrane is blocked by application of 2.4-dinitrophenol or by oxygen depriviation (Stieve, 1964; Baumann and Mauro, 1973). When the active sodium/potassium transport is blocked by ouabain, activability is lost when the prestimulus membrane potential (PMP) is still 40% of its original value (Smith et al., 1968; Stieve, 1974).

2. A certain membrane potential is a prerequisite for the activability of the light channels. Sustained depolarization of the membrane potential to zero by high external potassium causes a delayed loss in the ability of the light channel to be activated by light. A certain membrane potential and possibly a functional sodium/potassium

transport system is necessary to enable the membrane to undergo light induced conductance changes. It is suggested that the membrane potential probably creates and sustains an ordered structure of membrane constituents of dipole character which seems to be essential for the ability to conductance changes.

Possible dipolar membrane constituents responsible for this effect could be ionic channel forming molecules (probably not rhodopsin, since the early receptor potential does not disappear when the cell is depolarized for 2 h) or the Na/K-ATPase; this assumption could explain both the effect of depolarization and that of ouabain. The loss of activability is not directly due to the depletion of ATP, since ouabain, which does not cause a depletion of ATP, has the same effect as O_2-deficiency.

3. The activability of light channels depends on balanced ion concentrations:

a) Rising of the external calcium concentration $[Ca^{2+}]_{ex}$ causes an increase of membrane resistance R_d in the dark; if $[Ca^{2+}]_{ex}$ is very high (> 250 mM) the light response is reversibly abolished.

b) Lowering of $[Ca^{2+}]_{ex}$ causes membrane potential and membrane resistance R_d in the dark to decrease. With decreasing $[Ca^{2+}]_{ex}$ the PMP is gradually changed towards a positive value; if $[Ca^{2+}]_{ex}$ is very low (< ca. 10^{-9} M) the PMP is about +10 mV, which is in the vicinity of the saturation potential of the light response (the reversal potential of the light induced membrane current). With decreasing $[Ca^{2+}]_{ex}$ the light response becomes gradually smaller and disappears (after 10—20 min stay) in $[Ca^{2+}]_{ex} < 10^{-9}$ M.

Extracellular ionized calcium is necessary in a concentration $[Ca^{2+}]_{ex} > 10^{-9}$ M for the ability of the light channels to be activated by light. The observed effects would be expected if in very low $[Ca^{2+}]_{ex}$ the light channels were open permanently in light and in the dark.

The effects of lowering $[Ca^{2+}]_{ex}$ on membrane potential PMP, light induced membrane conductance change and receptor potential can be counteracted by additionally lowering $[Na^{+}]_{ex}$ (Stieve, 1974). According to Brown and Ottosen (1975) the supressive action of extracellular calcium ions on the light induced conductance increase (activation of light channels), is antagonized by K-ions.

These facts lead to the assumption of Ca- and Na- (and K-) ions competing for negatively charged (binding) sites at the cell membrane. The binding sites control the membrane conductance and especially the activation of the light channels: The more calcium is bound, the lower is the activability — and correspondingly, the more sodium (the less calcium) is bound, the higher is the activability; if only sodium is bound the light channels are permanently open.

Location of Conductance Controlling Binding Sites

The assumption that the dark permeability of the visual cell membrane for calcium ions is small leads to the conclusion that binding sites for Ca^{2+} which control the membrane conductance are located at the external surface of the cell membrane. Also the dependance of the excitability from external potassium concentration (Brown, 1976) favours the assumption of location of the sites at the external surface, since $[K^{+}]_i$ does not change much under these conditions.

Experiments from Lisman and Brown (1972), Lisman and Brown (1975a, b), Brown and Blinks (1974), Fein and Lisman (1975), and Fein and Charlton (1975)

and Brown (1977) show a strong influence of $[Ca^{2+}]_{in}$ on the light induced conductance change of the cell membrane of the ventral nerve photoreceptor of *Limulus*. They strongly indicate that calcium ions acting at the inner surface of the visual cell membrane also reduce the activation of light channels. Probably calcium ions acting at the outer or at the inner surface of the cell membrane have in some respect a qualitatively similar action on the activation of light channels; i.e. binding sites on both sides of the cell membrane probably influence the activability of light channels in the same direction.

Calcium Substitutes

Mg^{2+} can replace Ca^{2+} for this conductance controlling role, but with a weaker effect and not in all respects. In 100 mM $[Mg^{2+}]_{ex}$ membrane potential PMP and receptor potential amplitude are about 50% of that in 10 mM $[Ca^{2+}]_{ex}$. Therefore one can conclude that calcium exerts the described influence by binding to the cell membrane rather than by screening effect.

Lanthanum ions bind more strongly than calcium ions to the negative binding sites at the outer surface of the cell membrane. 1 mM $[La^{3+}]_{ex}$ lowers the dark permeability and blocks the light induced transient activation of the light channels in *Astacus*. Surrounding glial cells in *Limulus* may hinder the access of the La^{3+} ions to the visual cell membrane (Stieve et al., 1976).

The observed effects of $[Ca^{2+}]_{ex}$ and $[Na^{+}]_{ex}$ on the excitability of the light channel and on the dark conductance of the cell membrane can be explained in two possible ways:

a) Permanent fixation of calcium to a certain fraction of the binding sites of the membrane surface is a necessary prerequisite for the function (opening and closing) of the light channels. The amount of fixed calcium depends on $[Ca^{2+}]_{ex}$, $[Na^{+}]_{ex}$ and $[K^{+}]_{ex}$. When too many sites are occupied by calcium the light induced opening of the light channels is suppressed; when too few calcium ions are bound, the light channels are permanently open.

b) A transient release of Ca^{2+}, which is bound to the external membrane surface (and which can be replaced by Na^{+} or K^{+}) is a causal step for the activation of the light channel: For instance, binding of calcium could cause the light channels to close, whereas replacement of the bound calcium by binding of sodium could cause opening. The light stimulus could cause a transient affinity change of the negative binding sites, reducing transiently the preference for calcium over sodium.

Binding of Na^{+}, $K,^{+}$, Ca^{2+}, Mg^{2+} or La^{3+} to negative surface charges causes different changes in the surface potential, resulting in different potential profiles through the visual cell membrane. In nerve membranes there is good evidence to assume that Ca^{2+} acts on the excitability by changing the electrical field inside the cell membrane. If the same were true for the visual cell membrane, binding of calcium ions at the inner or at the outer surface of the visual cell membrane should have opposite effects on excitability since it has opposite effects on the internal electrical field of the cell membrane. Since calcium ions seem to have, in some respect, qualitatively the same action at both sides of the visual cell membrane, it does not seem likely that the electrical field inside the visual cell membrane is the main factor controlling the excitability of the light channels. Since the light channels, contrary to the sodium channels of the nerve membrane, are not activated by a membrane potential change but by a light induced change of the rhodopsin molecule, they seem to have no sensitive sensor triggered by the membrane potential.

Calcium ions act on the extracellular surface of the visual cell membrane via negative binding sites (for which they compete with sodium ions), which control the opening and the closing of the light channels. Probably by a similar mechanism calcium ions can control the activation of the light channels also at the inner surface of the visual cell membrane. However, according to considerations of Kramer (1975), the main effect of the above described changes in intracellular calcium ion concentration occurring during light adaptation seems to be the control of the gain of the amplification process which results in the opening of a great – and variable – number of light channels per photostereoisomerization of one rhodopsin molecule. Increasing $[Ca^{2+}]_{in}$ causes a decrease of the amplification factor (Fig. 1). This amplification may also be a membrane process.

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