ELECTRIC RESPONSE MECHANISM OF PHOTORECEPTORS

On the Mechanism of Electric Response of the Photoreceptors of the Barnacle and Other Animals

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Photoreceptors electric response mechanism

For the barnacle photoreceptors it is estimated that at 20 °C a half-maximal flash response requires the photic activation of 1% of the tubulous microvilli. The decrease of photo-electric efficiency at high intensities and low temperature is attributed to less efficient 2 quantum activation of microvilli. The estimate of the light induced charge flux yields evidence for ionic depletion in an extracellular space which is restricted by glia cells. The integration of the current during the transient of the response to steps of intense light yields limits for the extracellular volume which are in accord with electron microscopic results. The observed reaction scheme with a ratio of latency time : duration of response : adaptation time independent of temperature and type of photoreceptor is in a model explained with the same transfer processes occurring in volume elements of different size. A brief review of photoreceptors of other animals shows that in spite of different durations of the responses the reaction scheme of flash responses and the photo-electric efficiency (≈ 10^5 ions per absorbed photon) are similar for different photoreceptors.

The study of the transduction reaction in vision i.e. the enormous amplification of the energy of the absorbed light (input) into the electric energy of the receptor potential (output) requires a quantitative knowledge of the input-output relation. The photo-electric efficiency $\eta_q$ represents the charge flow which is caused by each absorbed photon. In a preceding publication $^2$ $\eta_q$ was estimated to about $10^5 e_o / (h \nu)_{abs}$ for the median and the lateral photoreceptor of the barnacle Balanus eburneus. It is the aim of this publication to compare these results with those obtained from other photoreceptors and to discuss the different phases of the electrical responses to flashes (receptor potentials) in regard to structures in the photoreceptor which were observed with the electron microscope at the median photoreceptor of the similar species Balanus cariosus $^3$. The consequences of the light induced reactions and ionic movements in the photoreceptors of the barnacle are to be discussed. It is also to be tested whether the conclusions which were drawn from the measurements at the barnacle photoreceptors can be applied in a similar fashion to results which were obtained at photoreceptors of other animals by other authors.

1. Photo-chemical reactions (input system)

It was shown $^2$ that the median eye of Balanus eburneus absorbs approximately $10^8$ photons at 21 °C if the flash stimulus has half-saturating intensity. About 10% of the volume of the photoreceptor consists in tubulus microvilli ($^3$, Fig. 4) which — according to the opinion of most investigators — contain the light absorbing photopigment. In order to ensure that excitation of the receptor membrane is accomplished only by photons and not by statistical (thermal) processes it appears plausible that the microvillus with a content of about $10^4$ photopigment molecules ($10^{-20} \text{cm}^3$/molecule of rhodopsin $^4$, 10% rhodopsin/microvillus) is the energy converting entity. The average volume of one microvillus is about $10^{-15} \text{cm}^3$ $^3$, the entire microvillus volume of the photoreceptor is approximately $10^{-7} \text{cm}^3$ $^3$. It therefore follows with the above assumptions that of the $10^8$ microvilli which are present in the photoreceptor about 1% absorb one photon each in order to produce a half-saturation response. On the basis of the assumption that maximum output efficiency is achieved only for single photon stimulation of one microvillus it becomes evident that flash stimuli which exceed the half-saturation intensity by two orders of magnitude lead to a participation of about 100% of the microvilli in photon absorption. Since light absorption is a statistical process many microvilli will at such intensities already have absorbed two photons. The output efficiency in regard to the incident quanta is for such high light intensities most likely to decrease.

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The observed temperature dependence of the flash energy required to produce a half maximal (= half-saturation) response with a factor of \(7 - 10\) for \(10^\circ\) temperature change \((Q_{10} = 7 - 10)\) could be explained with a “usual” \(Q_{10}\) of 2 – 3 of the chemical reaction of the microvilli and an additional saturation phenomenon due to multiple photon activation.

2. Electric response (output = charge flux)

The receptor potential is caused by the passing of ions \(\Delta n\) [moles] across the receptor membrane. Dividing the electrical charge flow \(\Delta q\) by the Faraday constant \(F \approx 10^6\) Asec/mole one obtains for \(\Delta n = i \Delta t / F\) moles, where \(i\) is the current and \(\Delta t\) the duration of an idealized rectangular response. Since the ions change place within volume elements \(V\) [cm\(^3\)] (on both sides of the receptor membrane) the concentration change \(\Delta c = \Delta n / V\) is given by:

\[
\Delta c = \frac{i \Delta t}{F V} \text{ moles cm}^{-3}.
\] (1)

With the approximate charge flow \(i \cdot \Delta t \approx 10^{-8}\) Asec (half-saturation response) and the total receptor volume \(V \approx 10^{-6}\) cm\(^3\) one calculates with eq. (1) a concentration change in the receptor of \(4 \cdot 10^{-5}\) mole/l. This light-induced concentration change is negligibly small compared to the usual intracellular concentrations of Na\(^+\) or K\(^+\) ions \((10^{-2} - 10^{-1}\) mole/l).

Electron microscopy revealed, however, that the receptor membrane is surrounded by a closely packed layer of glia cells of several \(\mu\)m thickness. The spacing \(1\) between glia cells and the receptor cells is in the order of 0.05 \(\mu\)m (3, Figs. 5, 6). Fig. 1 represents a schematic drawing of the mentioned morphology of the barnacle photoreceptors.

It was made probable that glia cells essentially have isolating properties in the optic nerve of Balanus\(^2\). If these cells would have the same function in the photoreceptor they would restrict the extracellular space of the receptor cells to the clefts between the receptor membrane (area \(A\)) and the glia cells, Fig. 1. The extracellular volume \(V\) of these clefts is then: \(V = A \cdot l\). The folding of the receptor membrane in the dendrites\(^3\) leads to an increase of surface area. If this increase were to quadruplicate the normal area the extracellular volume \(V'\) would be \(4 \cdot A \cdot l\). With \(A = 6.3 \cdot 10^{-4}\) cm\(^2\) of the median receptor of Balanus (Kr) one calculates \(V'\) to be \(1.3 \cdot 10^{-8}\) cm\(^3\). This is 1\% of the actual receptor volume. In this restricted extracellular space \(V'\) one calculates with eq. (1) a concentration change of \(4 \cdot 10^{-3}\) mole/l (for half-saturation). This concentration change is already in the range of a few percent of the usual ionic concentrations of biological fluids. It is evident that more intense illumination can lead to extracellular ionic depletion.

Such a depletion of ions is known to affect properties of the membrane such as the transmembrane potential drop and the resistance. The effect of an ionic depletion of the potential determining ion on one side of the membrane therefore leads to a decrease of the potential drop and an increase of the membrane resistance\(^5\). If light-induced ionic depletion would occur at photoreceptors one would expect an initial large depolarization (with unrestricted ion flow and small resistance) to be followed by a decrease of potential and paralleled by an increase of the receptor resistance.

At the lateral eye of Balanus and the ventral eye of Limulus Brown et al.\(^6,7\) and Millecchia et al.\(^8\), respectively, did indeed observe that at high light intensities the receptor potential is initially high and the membrane has a small resistance during this so called “transient”. During the steady level after the transient the potential is small and the resistance of the membrane is large. This steady level is obviously due to a constant ion supply probably by the glia cells.

On the basis of this depletion hypothesis it is possible to calculate with the current flow \(i\) and the duration \(\Delta t\) of the “transient” the depleting volume \(V_d\), provided the concentrations are known or assumed. With eq. (1) one calculates for the lateral eye of Balanus a depleting volume \(V_d\) of \(0.85 \cdot 10^{-8}\) cm\(^3\) or \(8.5 \cdot 10^{-8}\) cm\(^3\) for \(c = 10^{-1}\) or \(10^{-2}\) mole/l,
respectively \((i = 1.7 \cdot 10^{-7} A, \Delta t = 0.5 \text{ sec})\). Comparison with the volume which was calculated from histological data of \(V = 4.2 \cdot 10^{-6} \text{ cm}^3\) shows that these values of volumina are indeed with 0.5% and 5% of the total receptor volume in the expected range. Even through these estimates do not prove that the transient is a consequence of ionic depletion, they only show that such a process could be one possible explanation for the “transient”.

These estimates are in agreement with the findings of Zerbst et al.\(^9\) who discussed the restricted extracellular space as a chemical capacitor which influences the time constant of the photo response. This capacity per unit area \(C^*\) is given by:

\[
C^* = \frac{\Delta q^*}{\Delta E} = \frac{\Delta c \cdot F \cdot I}{\Delta E} \quad \left[ \frac{\text{Asec}}{\text{V cm}^2} \right] \tag{2}
\]

where \(\Delta c\) is the depleting concentration change, \(F\) the Faraday constant, \(I\) the thickness of the extracellular space and \(\Delta E\) the change of membrane potential due to depletion.

With the normal ionic concentrations of \(\Delta c = 10^{-2} - 10^{-1} \text{ M}\) and \(I = 1.5 \cdot 10^{-6} \text{ cm}\)\(^9\) or \(5 \cdot 10^{-6} \text{ cm}\)\(^3\) and \(\Delta E = 25 \text{ mV}\) one calculates for \(C^*\) the limits 2000 to 60 \(\mu\text{F/cm}^2\). This is an “interfacial” capacitor two to three orders of magnitude larger than the capacitor of the loligo nerve membrane\(^5\). Since the response is 1 msec and 100 msec for the spike of the loligo nerve and flash response of the Balanus photoreceptor, respectively the interpretation of Zerbst et al.\(^9\) is confirmed with the experimental findings. These estimates are confirmed by the measurements of Brown et al.\(^7\) who determined with the current clamp technique an “apparent” specific membrane capacity of 180 \(\mu\text{F/cm}^2\).

The discussion is certainly incomplete in so far as it does not include the ionic milieu of the photochemical microvillus system. Ionic concentration changes of this system could have similar effects.

It could also be possible that Na\(^+\) and K\(^+\) currents subsequently flow during the receptor potential. It is, however difficult to investigate this possibility because so little is known about the function of the glia cells which surround the receptor cells.

2.1. Equilibration processes

If ionic depletion is possible in photoreceptors the normal functioning of the visual process requires after the charge flux of the electric response equilibration processes in order to restore the “normal” concentration gradients. Because ionic reservoirs in cells do not have unlimited capacity certain energy consuming “pumps” (glia cells?) have to become operative in order to refill the volume elements. Ions will therefore have to be transported certain distances within the cells.

Short light flashes initiate a delayed and slow electric response (of 50 to 100 msec duration) the characteristics of which is independent of temperature: Time parameters such as latency time \(t_{\text{lat}}\), time to reach the maximum \(t_{\text{max}}\), total duration \(t\) and light-dark adaptation time \(t_{\text{ad}}\) are related by constant ratios with each other\(^2\). These ratios \(t_{\text{lat}} : t_{\text{max}} : t : t_{\text{ad}}\) are approximately the same for the two different photoreceptors of Balanus\(^2\). The total durations of the flash responses differ, however, by a factor of two for the two photoreceptors which were investigated\(^2\). It was assumed that this identity of reaction scheme and difference in reaction rate can be explained as being due to the same process occurring in compartments of different size. The receptor with the smaller compartments reacts in this model faster than the receptor with the larger compartments. The identity of reaction scheme would mean that the proportioning of all the compartments which participate in the reaction and their rates are the same for both receptors.

The transfer of energy or mass in the compartments could be rate determining for the light-induced reaction. It is evident that for the equilibration to the original state similar processes could control the rate.

In the simple case in which heat, energy or mass (particles) spread in an unrestricted space a characteristic length \(\lambda\) [cm] which is passed in a certain time \(\tau\) [sec] are correlated by:

\[
\lambda = \sqrt{D \tau} \quad \text{[cm]} \tag{3}
\]

where \(D\) [cm\(^2\)/sec] is the transfer coefficient. For diffusion a typical value of \(D\) for common ions in water such as K\(^+\) is about \(10^{-5}\) cm\(^2\)/sec.

Applied to the transfer processes in the compartments eq. (3) states that the reaction time \(\tau\) i.e. duration of the reaction of the latency period \(t_{\text{lat}}\) or the adaptation time \(t_{\text{ad}}\) are proportional to the square of the characteristic length \(\lambda\) of the corresponding compartments. If it is assumed that transfer coefficients for various photoreceptive processes are comparable in magnitude it is possible to make with the measured values of \(t_{\text{lat}}\) and \(t_{\text{ad}}\)
estimates of size of the corresponding compartments. If the adaptation e.g. the recovery of the photoreceptor after exhaustion by intense illumination requires transfer processes within the whole receptor the size of this "compartment" would be equal to the size of the photoreceptor; \( \lambda_{ad} = r \), where \( r \) is the half diameter of the receptor. With eq. (3) one can calculate the size of the compartment of the reaction of the latency period \( \lambda_{lat} \): For the same \( D \) one obtains:
\[
\lambda_{lat} = V_{ad} : t_{lat}, \text{ eq. (3)}.
\]

Insertion of the measured parameters of the median photoreceptor of the barnacle with \( r = 30 \mu m \), \( t_{ad} = 1.5 \text{ sec} \), \( t_{lat} = 27.9 \text{ msec} \) yields \( \lambda_{lat} \) to \( 4 \mu m \). This value is in a similar range as half the diameter of the microvillus zones of the median photoreceptor of the similar species balanus cariosus (3, Fig. 4). It remains to be proved that the microvillus zones of the lateral photoreceptor are larger than the microvilli of the median photoreceptor. According to the above hypothesis they should have a diameter of about \( \sqrt{2} \) times larger than the median, so \( (\lambda_{lat})_{\text{lateral}} \approx 5.7 \mu m \).

The observed temperature dependence of the time course of the flash-induced receptor potentials with a \( Q_{10} \) of 2—3 for the characteristic time parameters is an indication of the chemical nature of the transfer process. Diffusion controlled reactions should have a much smaller temperature coefficient.

3. Comparison of flash responses of Balanus with those of other animals

It is an important question whether the observed dependence of flash response parameters on the size of the two eyes of Balanus also apply to photoreceptors of other animals; i.e. whether the observed photo-electric processes are of general applicability in the visual process.

A comparison of experimental results in table I shows that — although crude assumptions had to be made — the photo-electric efficiency \( \eta_q \), eq (15) is for the considered photoreceptors of similar magnitude, i.e. in the order of \( 10^{14} \text{ Asec/(hr \text{abs})} \) which is equivalent to \( 0.6 \cdot 10^{6} \) univalent ions per absorbed photon. The discrepancy of No. 3 (Balanus) may be due to the fact that \( \eta_q \) was — in contrast to \( \eta_q \) in No. 1 and 2 — calculated for steady light stimulation. The comparison of the photo-electric efficiencies therefore shows that this important parameter of the visual process is similar in magnitude for different animals such as Aplysia and rat.

<table>
<thead>
<tr>
<th>No.</th>
<th>animal</th>
<th>( \theta ) °C</th>
<th>( t_{lat} ) 0.5 [msec]</th>
<th>( t_{lat} : t_{lat} )</th>
<th>( (dE/dt)_{0.5} ) [V/sec]</th>
<th>( \eta_q ) [Asec/hr \text{abs}]</th>
<th>author and remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Balanus med</td>
<td>21</td>
<td>19</td>
<td>1 : 5.4 : 54</td>
<td>1.2</td>
<td>( 2 \cdot 10^{-14} )</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Balanus lat</td>
<td>21</td>
<td>38</td>
<td>1 : 3 : 165</td>
<td>0.5</td>
<td>( 2 \cdot 10^{-14} )</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Balanus lat</td>
<td>20—25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>( 2 \cdot 10^{-16} )</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Apis</em></td>
<td>23</td>
<td>15</td>
<td>1 : 6.3 : 300</td>
<td>0.65</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Limulus lateral</td>
<td>9</td>
<td>170</td>
<td>1 : 3 : 230</td>
<td>0.13</td>
<td>( 4.4 \cdot 10^{-14} )</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Limulus ventral</td>
<td>20—25</td>
<td>50</td>
<td>1 : 5.5 : —</td>
<td>0.5</td>
<td>( 10^{-14} )</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Loligo</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>( 9.2 \cdot 10^{-14} )</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Aplysia</em></td>
<td>14</td>
<td>500</td>
<td>1 : 4.5 : —</td>
<td>0.09</td>
<td>( 1.1 \cdot 10^{-15} )</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>rat</em></td>
<td>33—35</td>
<td>10(? )</td>
<td>1 : 5 : — (? )</td>
<td>0.5(?)</td>
<td>( 3.7 \cdot 10^{-13} )</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Parameters of flash responses of photoreceptors of various animals.
The comparison of time-dependent response parameters in Table I shows that the reaction schemes of electric flash responses are similar for various photoreceptors: The characteristic response parameters such as latency time $t_{\text{lat}}$, total duration $t_t$, adaptation time $t_{\text{ad}}$, i.e. the ratio $t_{\text{lat}}:t_t:t_{\text{ad}}$ are similar for all the investigated photoreceptors. Long latencies are usually followed by relatively low values of the potential increase with time. So, apparently many photoreceptors obey the same photoreceptive reaction scheme even though the standard parameter of the photo-chemical reaction, the latency time $t_{\text{lat}}$ at half saturation, differs by as much as a factor of 50, see $t_{\text{lat}}$ of No. 8 and 9. A detailed morphological comparison of the structural elements which contain the photo-pigment would certainly be of interest. According to the model 2 the receptors with the longer latency should have the larger compartments. For the eye of Aplysia the length of the rhabdomers 10 (corresponding to the microvillus zones of Balanus of the diameter of about 4 μm) is about 20 μm. Insertion of the half-saturation latency $t_{\text{lat}} = 500$ msec and the dimension $\lambda = 20$ μm in eq. (3) yields for this eye approximately the same “transfer coefficient” as for Balanus. The underlying processes therefore seem to be the same for both eyes. This fact is also documented by the observation that the dependence of the latencies on the light intensity is for the considered photoreceptors very similar provided they are normalized to the same excitation. So is $d(t_{\text{lat}})/d \log I$ for many photoreceptors i.e. Limulus ventral eye 8 Limulus lateral eye 11 Balanus 6 and Aplysia 10 in the range of 30 – 50% per decade of light intensity ([$t_{\text{lat}}$]$_{0.5}$ = 100%). Jacklet 10 observed (in accordance with the observations at Balanus) a temperature dependence of 2 – 2.5 per 10° for the latency time of the eye of Aplysia.

Conclusion

The similarity of photo-electric efficiency and time course of flash induced receptor potentials of different photoreceptors might serve as indication for a common mechanism of the primary electric response of these photoreceptors. It remains to be tested experimentally whether the receptor length constant $\lambda_\text{r} [\text{cm}]$ – which is defined as the ratio of photopigment volume to excitable membrane area 1 – is the rate determining parameter in vision. $\lambda_\text{r}$ would be analogous to $\lambda_\text{n}$, the length constant of a nerve. For both types, visual and nervous excitation volume and surface properties would thus characterize the rate. Only intense studies of morphology and function of photoreceptors will show to what extent this generalization is valid. It also remains to be investigated whether there is in photoreceptors a “tuning” of the receptor length constant and the effective interfacial capacitance of extracellularly restricted space in such a manner that a large length constant would require a large capacitor (large extracellular space) in order to become operative in the most efficient manner.