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# Discussion* to III. Conductivity Control of the Visual Cell Membranes

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## Introduction

In this session on the conductivity control of the receptor cell membrane we are concerned with

a) the mechanism by which the information contained in the excited photopigment molecule is transmitted to the receptor outer membrane,

b) the conductance changes generated there,

c) subsequent adaptation processes (but only those which involve modulation of membrane conductance).

One particularly useful concept is that the coupling between photochemical event and the electrical response of the membrane is by the release of a diffusible intracellular transmitter molecule. Unfortunately the photopigment transition responsible for the release of "transmitter" is unknown and there are many unexplained features about the conductance change at the membrane.

The conductance changes in which we are interested involve both the "opening" and "closing" of "channels" in the membrane. There is *no* candidate that I know of for the transmitter of the "opening" process and very few candidates for the "closing" process. Since the "closing" process is one of adaptation in invertebrates and excitation in vertebrates the discussion had best be divided into two sections beginning with the studies on invertebrates.

## Invertebrate Receptors

### *The Role of Intracellular Ionized Calcium in the Regulation of Receptor Sensitivity*

Brown reported that in the ventral eye of *Limulus,* the intracellular calcium concentration, as estimated by the calcium-indicator Arsenazo III, may increase to as

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much as 0.5 mM in light adapted cells (with very strong lights). In dark adapted cells, however, the $Ca^{2+}$-concentration is approx. 4 decades lower.

Injection of calcium into the receptor cells reduces their sensitivity, and reduces the time to peak.

Duncan pointed out that in Sepiola the fast part of the photoresponse gets slower in high calcium saline.

Stieve said that in *Limulus* the response sometimes becomes slower and sometimes faster after external application of high $Ca^{++}$ concentrations.

Meech said that injection of calcium into *Helix* neurones produces a fall in the intracellular pH ($pH_i$). In *Balanus* photoreceptors a small decrease in $pH_i$ upon illumination is measured using pH sensitive electrodes inserted into individual receptor cells. An equivalent $pH_i$ change induced in $CO_2$-saturated saline gives a large decrease in the sensitivity of the receptor. Thus calcium injection may act on the receptor sensitivity via a reduction in $pH_i$.

Bonting pointed out that the pH change could result from calcium/proton exchange by the mitochondria. Meech agreed.

However, Brown said that injection of pH buffers (in the pK range 6.3—7.5) into *Limulus* photoreceptors while reducing the time to peak of the photoresponse to brief light flashes had no significant effect on the size of the receptor potential. He said, however, that he had not tested the effects of longer test flashes and could not rule out the possibility that $pH_i$ changes may be involved in slow processes.

Clayton asked about the difference between the time course of the change in intracellular calcium as measured by aequorin and the time course of the receptor potential.

Brown replied that experiments with low concentration of Arsenazo III demonstrate that during the receptor potential the Ca-concentration of free $Ca^{++}$ increases, but the maximal Ca-transient follows the maximal voltage transient of the response and that in a strongly light-adapted photoreceptor $Ca^{++}$ remained high for a long time. High concentration of the dye show effects on the response similar to EDTA, eliciting a prolonged receptor potential.

Brown concludes from these experiments that $Ca^{++}$ is probably a transmitter for adaptation and not for excitation.

Meech said that in many other tissues an increase in intracellular calcium rather than affecting the sodium conductance leads to an increase in potassium conductance.

### *Conductance Changes in Invertebrate Receptors*

Muiser presented experiments which show that in the photoreceptor of the fly the conductance does not decrease after the transient of the receptor potential (ReP), furthermore he could demonstrate that under current clamp transient and plateau exhibit different reversal potentials. Muiser proposed that the ReP is composed of two processes a fast one which occurs transiently at the onset of illumination and a slower one which remains as long as the light is on.

Brown pointed out that voltage clamp experiments on *Balanus* and *Limulus* photoreceptors did not support this suggestion since in these receptors all phases of the receptor potential have the same reversal potential.

Thurm described the situation in mechanoreceptors of insects where the reversal potential of the receptor potential changes during adaptation although the total conductance does not.

Minke, quoting work by Hanani and Shaw, said that in *Balanus* the undershoot between transient and plateau of the receptor potential when examined under current clamp appeared to be caused by an increase in potassium conductance.

Hillman quoted work by Gorman and McReynolds on the hyperpolarizing receptor potential in *Pecten* which indicates that it is due to an increase in permeability to potassium possibly caused by an increase in $Ca^{++}{}_i$.

Brown said that there are clearly great difference between different photoreceptors. In *Limulus*, for example, calcium appears to be released internally whereas there is no evidence that this occurs in *Balanus*. In *Limulus* the reversal potential of light-induced current is independent of external $Ca^{++}$ if the external sodium concentration is low. In *Balanus*, however, the reversal potential depends on $Ca^{++}$ under similar conditions.

Baumann asked if there was evidence for transmitters other than $Ca^{++}$ or $H^+$ – small organic molecules for example.

Korenbrot said that the level of cyclic GMP is light sensitive in vertebrate retinae but the rate of change appears to be slow (ca. 10 s).

## Vertebrate Receptors

Kramer bridged the gap between the vertebrate and invertebrate section of the discussion in the following way.

"Although the receptor potentials of invertebrate and vertebrate photoreceptors differ considerably, I would like to direct attention towards the similarities between the *adaptive* process in invertebrates and the *excitatory* process in vertebrates:

a) Both processes reduce the conductivity of channels whose reversal potential is around 10 mV.

b) In both cases return to the dark-state occurs at successively slower rates the stronger the preceding light signal.

c) Intracellular calcium may play a similar role in both cases.

Thus I suggest that the bump-processes which are stimulated by photon absorption in invertebrates take place continuously at a fixed rate in vertebrates. Light adapts the bumps and leads to hyperpolarization. The hypothesis is consistent with the finding that in turtle cones the voltage fluctuations decrease under illumination [Simon, E. J., Lamb, T. D., Hodgkin, A. L.: Nature (Lond.) **256,** 661 (1975)].

### *The Significance of "Noise" Measurements in Turtle Cones*

According to Lamb the electrical noise in turtle cones is due to random opening and closing of ion channels in darkness at a residual intracellular $Ca^{++}$ concentration. It is not clear whether this results from fluctuations in the intracellular $Ca^{++}$ level or from random binding at an approximately fixed concentration. In either case though, Lamb estimates that a substantial fraction of ion channels are closed in darkness. In addition, the time integral of the noise event (100 $\mu$V $\times$ 40 ms) is very

similar to that of the photon event (25 μV × 150 ms), suggesting that the channel closures involved in the two cases might be the same.

At the request of Conti, Lamb reported some experiments on the voltage dependence of noise saying that the noise decreased both on hyperpolarization and on depolarization. Lamb mentioned that there are many technical difficulties in performing experiments with current injection. There is, however, some evidence that the decrease in noise seen during illumination is not solely due to hyperpolarization. He proposed that either binding or removal of the molecule which blocks ion channels, e.g. $Ca^{++}$, might be voltage dependent.

Conti then said that the decrease in membrane voltage noise during bright illumination of turtle cones is also associated with a large membrane hyperpolarization. It seems difficult in these circumstances to rule out the possibility that at least some of the noise change is merely due to a decrease in the driving force across noisy channels which are light insensitive. This is an additional complication to the fact that voltage dependent channels could also exist in the membrane and that it is fairly arbitrary to translate voltage-noise into current-noise spectra in the absence of simultaneous reliable measurements of membrane impedance.

*Properties of Rod Discs*

As a test of the hypothesis that some transmitter molecule is released by the discs in the rod outer segment, Thurm asked: "Has somebody checked the receptor potential latency of a rod photoreceptor as a function of the radial distance between the outer segment discs and the cell membrane surrounding the discs? This distance may be artificially changed by altering the osmotic pressure of the bathing solution".

During the morning session Korenbrot had suggested that a Ca-Mg activated ATPase pump like that in the sarcoplasmic reticulum, might operate in the rod sac membrane. Bonting provided evidence which pointed to a different system. He said that the Mg ATPase activity in rod outer segments is not increased by addition of $Ca^{++}$ ($10^{-6}$—$10^{-2}$ mM), but is increasingly inhibited. Ruthenium red and $La^{3+}$ ions, which inhibit sarcoplasmic Ca-Mg ATPase and calcium uptake, do not inhibit calcium uptake in rod sacs (Bonting and Daemen, 1976). Bonting also referred to unpublished work by Schnetkamp, Daemen and Bonting which showed that rods depleted of calcium take up calcium by an ATP-dependent process. Uptake is greatly reduced in the presence of 100 mM $Na^+$ which leads to the conclusion that the uptake system consists of a Ca-Na exchange carrier. In non-depleted cells uptake occurs through Ca-Ca exchange and is ATP-independent.

At the end of the discussion the comments by Hofmann touched on several of the points mentioned by previous speakers: "We have studied the rapid changes in light scattering from preparations of bovine rod outer segments following illumination. One of the light-induced changes in structure which are revealed by this technique is a very small, fast step decrease in disc thickness (signal P). It is proportional to the exciting flash intensity for flashes bleaching not more than 2% rhodopsin. Kinetic analysis reveals that the transition from metarhodopsin I to metarhodopsin II is the trigger reaction for the process producing signal P. However, only meta I molecules arising from lumirhodopsin are effective not those in equilibrium with

meta II although there is no difference between the two species that can be detected by absorption spectrophotometry in the visual range. The disc shrinkage does not reflect an osmotic effect and consequent decrease in the ionic gradient across the disc membrane.

On the basis of experiments at different pH we suggest that the probable molecular origin of the change in disc thickness arises from an active shift of a proton into the inner disc phase.

There is no recovery in this system but the amplitude of the response is not proportional to the content of unbleached rhodopsin. It may be that the rhodopsin is interacting in rapidly changing pools. In collaboration with F. Siebert und R. Mull it has been possible to separate the rhodopsin protein band into 3 functionally interacting components.

Concerning the role of calcium ions we have loaded bovine disc vesicles with calcium. Upon resuspension in isotonic Ca-free saline and illumination a change in light scattering gave a measure of the change in disc size and indicated ion movement. The amplitude of this lightscattering change, which was not seen in discs loaded with $MgCl_2$, NaCl or KCl under the same conditions, was proportional to the level of unbleached rhodopsin. The effect is not seen if the discs are suspended in high sodium saline. However, the effect is too slow to be involved in excitation (half time 500 ms) and a role of $Ca^{++}$ in adaptation appears to be more likely".