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Phototransduction and light-iinducted mitochondrial activation in blowfly compound eyes

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Chapter 6

introduction

The present thesis considered two aspects of phototransduction in the blowfly *Calliphora* vicina mutant chalky. First, we studied the mechanism of the basic process, conversion of light to an electrical signal. And second, we examined the light-induced activation of mito-chondrial respiration, necessary to support phototransduction.

two Ca²⁺-pathways in blowfly phototransduction

In Chapter 2 we have studied the role of the extracellular calcium concentration on the biochemical transduction of absorbed light to a receptor response. For that purpose we have developed a semi-intact preparation, in which the retina can be superfused with various solutions. Exposing blowfly photoreceptors to a medium containing 10 mM EGTA and no added Ca^{2+} ($[Ca^{2+}]_e$ approaches 1 nM), has a two-fold effect on the light-induced receptor potential. First, the kinetics of excitation is slowed down to such an extent that the peak in the receptor potential is fully suppressed. Second, the receptor potential does not remain depolarized during prolonged illumination, but decays within a few seconds to the resting membrane potential, so that light-off is no longer visible.

Exposing the retina with Ringer containing the Ca^{2+} -entry blocker Co^{2+} , yields only part of the effect of lowering $[Ca^{2+}]_e$. That is, the kinetics of excitation is slowed down, but the receptor potential does not decay to the resting membrane potential during ongoing illumination. Interestingly, it has been shown that another Ca^{2+} -entry blocker lanthanum, La^{3+} , induces a decay of the receptor potential, whereas the peak of the receptor potential remains relatively unaffected (Hochstrate 1989). Therefore we suggested in Chapter 2 that two Ca^{2+} -entry pathways are operational during blowfly phototransduction. Possible roles for these Ca^{2+} -entry pathways were discussed.

light-induced mitochondrial activation

In Chapter 3, 4 and 5 we have examined the respiratory metabolism. STAVENGA and TINBERGEN (1983) and TINBERGEN and STAVENGA (1986, 1987) have shown that when a dark-adapted blowfly is suddenly intensely illuminated, the flavoproteins in the photoreceptor cells are transiently oxidized. In Chapter 3 we have presented measurements of the redox state of other mitochondrial pigments, namely NAD and cytochrome b, c and aa₃. We have measured the light-induced change in the redox state of NAD using its fluorescence properties (see also Chap-

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In Chapter 4 tivation was the flavopro ties, that al cussed that

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Summary

ter 5). It appeared that following 2 min dark-adaptation, NAD is oxidized within 2 s after the onset of the illumination. In most flies, the NAD redox state does not shift back, in contrast to the flavoproteins. The redox state of the cytochromes was studied by measuring absorbance changes in the compound eye. We have established absorbance difference spectra, *ie.* the absorbance in a dark-adapted retina *minus* the absorbance of a light adapted one. The spectra showed that cytochrome b is oxidized after the onset of illumination. The timecourse is in most flies virtually monophasic, *ie.* cytochrome b is oxidized within 2 s after the onset of illumination, similar to the NAD redox state.

Apparently, the mitochondrial activation does not only consist of a transient oxidation of flavoproteins. Our data suggested that several or perhaps all electron carriers in the mitochondrial respiration are oxidized when a dark-adapted compound eye is suddenly intensely illuminated. It was suggested in Chapter 3 that the stimulatory effect must be directed at enzymes located on the O_2 -side of cytochrome b. It is not likely that a decreased ATP/ADP ratio causes the observed oxidation of the mitochondrial pigments; therefore a role for $[Ca^{2+}]_i$ is hypothesized (TSACOPOULOS *et al.* 1983; CoLES *et al.* 1984; see introduction). Accordingly, we proposed in Chapter 3, that a protein exists as in vertebrate mitochondria, that, unless it is bound by Ca^{2+} , inhibits the ATP-synthetase.

receptor potential and mitochondrial activation

In Chapter 4 the relation between phototransduction and the light-induced mitochondrial activation was further characterized. Simultaneous measurements of the receptor potential and the flavoprotein fluorescence showed that the mitochondria are activated by light intensities, that also induce a peak-to-plateau transition in the receptor potential. We have discussed that this result supports the Ca^{2+} -hypothesis.

Furthermore we demonstrated in Chapter 4 that the blowfly *Lucilia cuprina* mutant w^F also shows light induced mitochondrial activation. In addition, we have examined the mitochondrial activation in the phototransduction mutant *Lucilia cuprina* w'nss, which receptor potential has no steady state. The time course of the transient change in flavoprotein redox state was different from the one in normal white-eyed flies; as expected, it resembled the pulse-response of the flavoprotein fluorescence, that was presented in the same Chapter.

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Chapter 6

fluorescence properties of NAD and flavoproteins

In Chapter 5 we have inspected the NAD and flavoprotein fluorescence properties more closely. We recall, that NAD fluorescence is excited with UV and measured in the blue, whereas flavoprotein fluorescence is induced with blue light and recorded in the green. In Chapter 5 we examined the question whether changes in NAD fluorescence also include a contribution of the flavoprotein fluorescence, and *vice versa*. We have presented emission difference spectra that suggest that NAD and flavoprotein fluorescence can indeed be measured independently.

Thus we have investigated the specific case how hypoxia affects the NAD and flavoprotein redox state. The preliminary data suggest, that upon the onset of hypoxia NAD is markedly reduced, but does not remain fully reduced during 30 min hypoxia, whereas the flavoproteins remain reduced throughout the hypoxia. The possibility of the existence of a form of anaerobic metabolism has been discussed.

CHAP

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