Cytochemical Localization of Guanylate and Adenylate Cyclase in Photoreceptor Cells of the Fly

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In photoreceptor cells of invertebrates light triggers an enzyme cascade in which the phosphoinositide pathway is crucially involved. Likewise, there is growing evidence of an important role of cyclic nucleotides, too. To localize these enzymes able to catalyze the formation of cGMP and cAMP, the spatial distribution of guanylate cyclase (EC 4.6.1.2) and adenylate cyclase (EC 4.6.1.1) was determined in photoreceptor cells of the fly. In photoreceptor cells of the blowfly (*Calliphora erythrocephala*), the electron dense reaction product of guanylate cyclase was found within the phototransducing region, the rhabdomeral microvilli and in the mitochondria. Staining was also observed throughout the cytoplasm of the microvilli. With the same cytochemical method, reaction product for adenylate cyclase was found on the tips of the photosensory membrane, and not in the cytoplasm of the rhabdomeral microvilli. The results presented here further argue for an important role of one or possibly two cyclic nucleotides in the photoreceptor cells, and possibly in the process of phototransduction of invertebrates.

Introduction

For invertebrates, the phosphoinositide pathway plays a crucial role in the phototransduction mechanism. A number of studies using biochemical or electrophysiological methods have shown that the production of inositol trisphosphate followed by the release of Ca^{2+} from intracellular stores is stimulated by light, and that this pathway is involved in the opening of ion channels (for review see Selinger *et al.* 1993).

In addition, an essential role for cyclic nucleotides in light-stimulated opening of ion channels seems likely. Intracellular injection of cGMP or its poorly hydrolysed analogue 8-Br-cGMP into *Limulus* cells could produce depolarizing membrane currents (Johnson *et al.*, 1986). Similarly, Feng *et al.* (1991) excited photoreceptor cells by the injection of the synthetic derivatives 8-Br-cAMP and 8-Br-cGMP, but not with cAMP or cGMP itself. Furthermore, ion channels in membrane patches excised from the light-sensitive lobe of *Limulus* cells could be activated directly by cGMP (Bacigalupo *et al.*, 1991).

With biochemical methods, a guanylate cyclase (GC) from squid photoreceptors was characterized, but no light-induced change in the activity could be detected (Robinson and Cote, 1989). Recently, Yoshikawa *et al.* (1993) isolated a *Drosophila* gene encoding a head-specific soluble GC which is enriched especially in the eye.

Conflicting results have been reported for changes of the cGMP level in response to illumination of retinal tissue of the squid. Saibil (1984) and Johnson *et al.* (1986) found a light-induced increase in cGMP, but no significant change could be detected by Brown *et al.* (1992) and Seidou *et al.* (1993).

Recently, Nagy (1993) showed that the injection of a phosphodiesterase (from the bovine heart) which is able to hydrolyse the cyclic nucleotides cAMP and cGMP, severely affects the light-response of *Limulus* ventral photoreceptors. The presence of a phosphodiesterase localized directly in the microvilli of photoreceptor cells of the fly was shown lately, too (Schraermeyer *et al.*, 1993).

As no data are available for the spatial distribution of guanylate and adenylate cyclase (AC) in photoreceptor cells of invertebrates, we thought it would be interesting to see whether there are GC and AC in those cells, and if so, in which parts of the cells enzymes are localized. We could show that cGMP and cAMP can be produced directly in the microvilli of photoreceptor cells of the fly.

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Materials and Methods

The preparation of retinal tissues of blowflies *(C. erythrocephala)* was carried out as described earlier (Schraermeyer *et al.*, 1993). Animals had been exposed to day light before preparation. For cytochemical localization of guanylate and adenylate cyclases the method of Saito (1977) and Saito *et al.* (1980) was used with slight modifications. Tissue samples were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 1 h. The incubation medium for adenylate cyclase consisted of 80 mM Tris maleate buffer, pH 8.5, 2 mM $MgCl_2$, 2 mM theophylline 0.5 mM 5′-adenylylimidodiphosphat (AMP-PNP) (Sigma, Deisenhofen, Germany), 2 mM lead nitrate and 7% sucrose. The incubation medium for guanylate cyclase consisted of 40 mM Tris buffer, pH 7.8, 2 mM theophylline, 3 mM manganese chloride, 0.5 mM guanylylimidodiphosphate (GMP-PNP) (Sigma, Deisenhofen, Germany) and 2 mM lead citrate. For comparison, the same media were used, but no nucleotide was added. Tissues were incubated for 2 h at 37 °C and were postfixed in 1% osmium tetroxide for 1 h, dehydrated in acetone and embedded in Spurrs resin. Ultrathin sections were poststained with uranyl acetate and lead citrate before examination in a Philips EM 300.

Results

In the photoreceptor cells of the blowfly electron dense reaction product of guanylate cyclase was found within the microvilli of the rhabdomeres (Fig. 1) and within the mitochondria (not shown). The enzyme seemed to be localized in the cytoplasmic part of the microvilli rather than attached to membranes. This is indicated by the fact that the cell membranes of the microvilli appeared electron lucent compared to the lumen of the microvilli (Fig. 1 and 2). The latter finding was particularly prominent in crossly sectioned microvilli of the rhabdomeres (Fig. 1b).

Quite another picture is obtained when the ATP-analogue is used instead of the GTP-analogue. The reaction product of adenylate cyclase was detected on the tips of the rhabdomeral microvilli (Fig. 3) whereas the lumina of the microvilli were not stained. Mitochondria did not contain reaction product of adenylate cyclase. Due to the low concentration of fixative in the histochemical preparations, the ultrastructure was not preseved as well as normally after routine electron microscopy. Without addition of the guanylate or adenylate analogue no reaction product was detected (Fig. 4).

Discussion

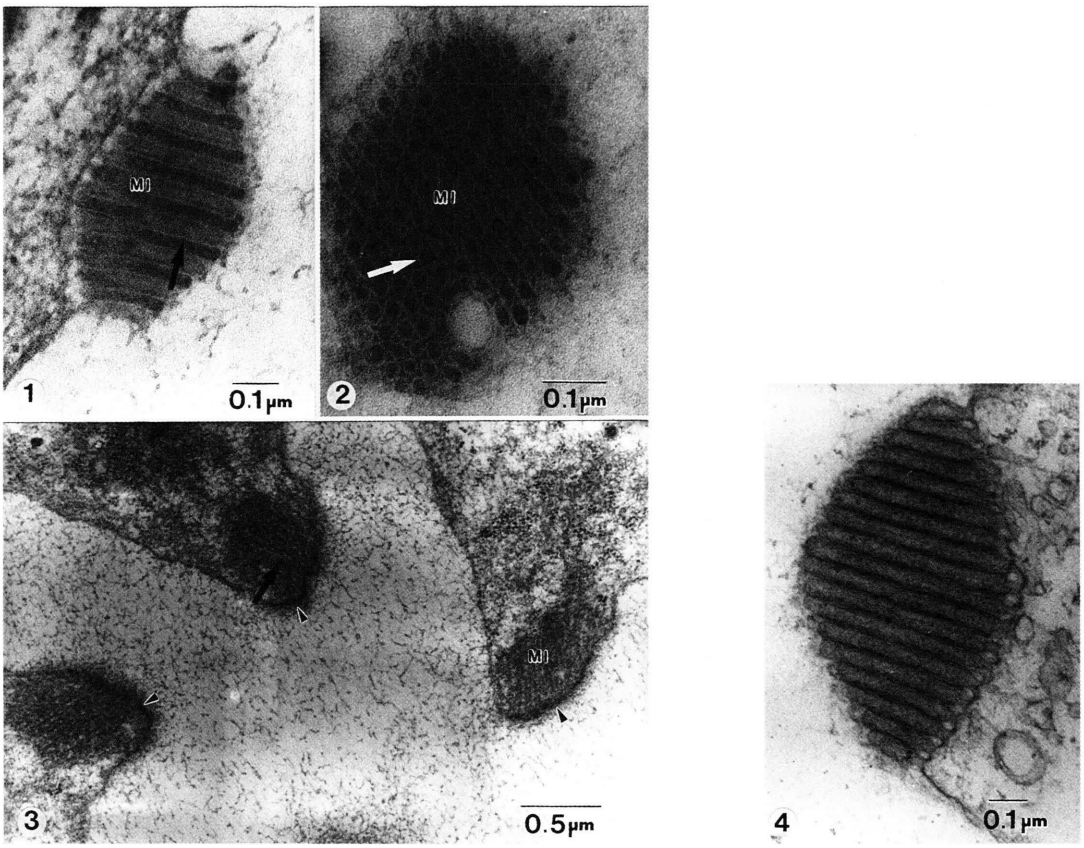
The results show that guanylate cyclase and adenylate cyclase activity are present in photoreceptor cells of the blowfly *Calliphora erythrocephala.* Moreover, both enzymes can be localized directly in the rhabdomeral microvilli. Recently the presence of a phosphodiesterase in those microvilli was demonstrated too (Schraermeyer *et al.*, 1993). Thus the enzymes for cGMP and cAMP metabolism are present directly in the phototransducing region of these photoreceptor cells.

There is a clear difference between the location of the reaction products of the GTP- and the ATP-analogue. As the nucleotides should not differ in their ability to diffuse, this indicates that the precipitates most probably reflect the distribution of GC and AC, respectively.

The reaction product for guanylate cyclase is located within the cytoplasm of the microvilli rather than in the membranes of the blowfly photoreceptor cells (see Fig. 1). This indicates that this GC belongs to the class of soluble guanylate cyclases.

Robinson and Cote (1989) determined GC-activity of photoreceptor membranes from the squid (*Loligo pealei*). GC activity was found to be membrane-associated exclusively. This difference to our results may be due to the different assay conditions used for histochemistry and for the biochemical assay or it may reflect a species distinction. Currently the latter interpretation seems likely, because in another fly (*Drosophila*), Yoshikawa *et al.* (1993) have demonstrated an enrichment of mRNA that encodes for a GC in retinal tissue and this GC is a soluble one too.

Our results give no answer to the question whether cyclic nucleotides exert their effects by the opening of those channels which are directly affected by the transmitter built by the light-driven enzyme cascade or whether these cyclic nucleotides act as modulators (e.g. for adaptation) in the process of phototransduction. The presence of enzymes for formation (GC and AC, this study) and



Figs 1 and 2. Localization of guanylate cyclase activity in the photoreceptor cells of the fly. Longitudinal section (Fig. 1) and cross section (Fig. 2) of microvilli. Electron dense reaction product of guanylate cyclase was found within the microvilli (MI) of the rhabdomeres. The enzyme seems to be localized in the cytoplasmic part of the microvilli rather than attached to the membranes, because the cell membranes of the microvilli appear electron lucent (arrow) compared to the lumen of the microvilli.

Fig. 3. Localization of adenylate cyclase activity in the photoreceptor cells of the fly. The reaction product of adenylate cyclase was detected close to the membranes of the tip of the rhabdomeral microvilli (arrowheads) whereas the lumina of the microvilli (MI) were not stained (arrow).

Fig. 4. This retina of the fly was treated as those for adenylate cyclase activity, however, the substrate (AMP-PNP) was omitted. Membrane bound or cytoplasmic electron dense reaction product of adenylate cyclase is lacking.

for degradation (PDE, Schraermeyer *et al.*, 1993) of cAMP and cGMP directly in the microvilli of the fly further argues for an important role of one or both cyclic nucleotides in the phototransduction of invertebrates.

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