Reversed Light Reaction of the Screening Pigment in a Compound Eye Induced by Noradrenaline

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Migration of the screening pigment in the compound eye of the sphingid moth *Deilephila elpenor* is altered by noradrenaline, as shown by microreflectometric measurements on eyes of intact moths and by transmission microscopy on preparations consisting of the screening pigment cells and dioptric structures. Local application of noradrenaline inverts the reaction of the pigment to light stimulation; light causes a contraction of the pigment instead of the normal dispersion. It is suggested that catecholamines are involved in the normal regulation of pigment migration.

Introduction

In the superposition eyes of sphingid moths the intensity of the light reaching the photoreceptors is regulated by movement of pigment granules located in specialized screening pigment cells (see [1–4]). In the dark-adapted eye the pigment is contracted distally between the crystalline cones, and much of the incident light is reflected by the baskets of tracheols that surround the photoreceptors in each ommatidium. The reflected light can be seen as an "eye glow" or a "pupillary response" (see [5]). Exposure to an adapting light causes the pigment granules to disperse, and the eye glow to disappear. In the sphingid moth *Deilephila elpenor* the spectral sensitivity of pigment expansion differs markedly from the spectral sensitivity of the retinula. The sensitivity of the retinula is maximal in the green region, λ_{max} = 525 nm, and has smaller maxima in the blue-violet, λ_{max} = 440 nm, and ultra-violet (UV), λ_{max} = 350 nm, regions of the spectrum [6]. In contrast, maximal sensitivity of the pigment expansion in *Deilephila* has been recorded in the UV region, a medium sensitivity in the blue-violet region, and a small sensitivity, about 0.01, in the green region close to the absorption peak of the numerous green sensitive visual cells [7, 8]. The dissimilarity between the spectral sensitivities of pigment migration and of the retinula suggests that in *Deilephila* pigment migration is possibly controlled by the activity of the UV and blue-violet sensitive photoreceptors. In other species the spectral sensitivities are more similar. In the related moth *Manduca sexta* the spectral sensitivity of pigment expansion matches the action spectrum of the retinular response [9].

Pigment migration can also be elicited in preparations consisting of only the screening pigment cells, dioptric structures and small visual cell rests (free from rhabdomes). The light-induced dispersion of the pigment in such preparations can be determined by transmission microspectrophotometry. The time course of change in transmission measured in the presence of potassium chloride is similar to the change in reflectance recorded in intact eyes [8]. It is therefore possible that the pigment cells, or the adjacent Semper cells, contain a photopigment, which can trigger movement of the pigment granules independently of photoreceptor activity. It may then be asked if pigment expansion is controlled only by a single photochemical mechanism, probably in the visual cells, or if it is controlled by an interaction between such mechanisms located in different types of retinular cells, *i.e.* in the visual cells and in the pigment or Semper cells. Changes in the extracellular ionic composition due to photoreceptor excitation seems not to be an essential component in such an interaction, since maximal excitation of the green sensitive photoreceptors elicits only a very small expansion of the screening pigment. A transmitter mediated interaction between the different cell types

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seems more likely. In addition to the UV-blue induced light reaction, a neuro-humoral regulation of pigment migration is further supported by the vast difference in reactivity of pigment migration during day and night. Catecholamines may be candidates for such cell communication modulating the pigment migration, as indicated by the results of the study reported here.

Materials and Methods

The technique for measurement on intact eyes of *Deilephila* and on the eye preparations consisting of only peripheral cells and visual cell rests has been described in detail [8] and is only briefly summarized here. The eyes were cut tangentially to the cornea distally to the rhabdomes. The preparation was placed on an agar gel containing potassium chloride, 200 mM, and glucose, 50 mM, and kept in a plexiglass-chamber covered by a cover glass to ensure constant ion concentration in the supporting gel and constant humidity in the chamber. A small droplet of potassium chloride solution containing the drug to be tested was dropped onto the agar gel. The effect on the pigment migration of the following drugs (10 mM solutions) was tested: Dopamine, D,L-noradrenaline, D,L-adrenaline, D,L-isoprenaline, dichloroisoproterenole and phenoxybenzamine. In the experiments on intact eyes contact gel (Siemens) containing the drug was applied on the cornea. The contact gel made the cornea transparent to ions, and also to small drugs, as shown by the experiment illustrated in Fig. 2. Reflectometric measurements were made using a set-up based on a Leitz Orthoplan fluorescence microscope. Pigment migration was stimulated by monochromatic or broad band light, and the eye glow reflected from the tracheols within the stimulated area (diameter 30 to 40 corneal facets) was monitored microphotometrically by a red measuring beam. Two crossed polarization filters eliminated light reflected from the cornea. Transmission microphotometry was made using the same microscope set-up. Stimulating lights (2 to 4 s) usually were a monochromatic blue stimulus (λ_{max} = 430 nm; monochromator Bauer BM, bandwidth 2 nm; maximal intensity = 3.4×10^{15} photons $\times$ cm$^{-2} \times$ s^{-1}), a broad band UV stimulus (λ_{max} = 370 nm; filter Schott UG11; half bandwidth about 80 nm; maximal intensity = 2.4×10^{16} photons $\times$ cm$^{-2} \times$ s^{-1}), or a blue stimulus (λ_{max} = 445 nm; filters Schott BG12, GG435 and KG1; half bandwidth about 50 nm; maximal intensity = 3×10^{16} photons $\times$ cm$^{-2} \times$ s^{-1}). The red measuring light came from a variable tungsten lamp (6 V; 5 A) fitted with an edge filter (Schott RG630; 4 mm).

Results and Conclusions

The variation in time course of change in eye glow with stimulus intensity in an intact animal is seen in Fig. 1a. The eye glow was measured as the number of photons per 10 ms reflected from the tracheolar layer. The light stimulus, after a latency of about 20 to 30 s, was followed by a decrease in reflectance.

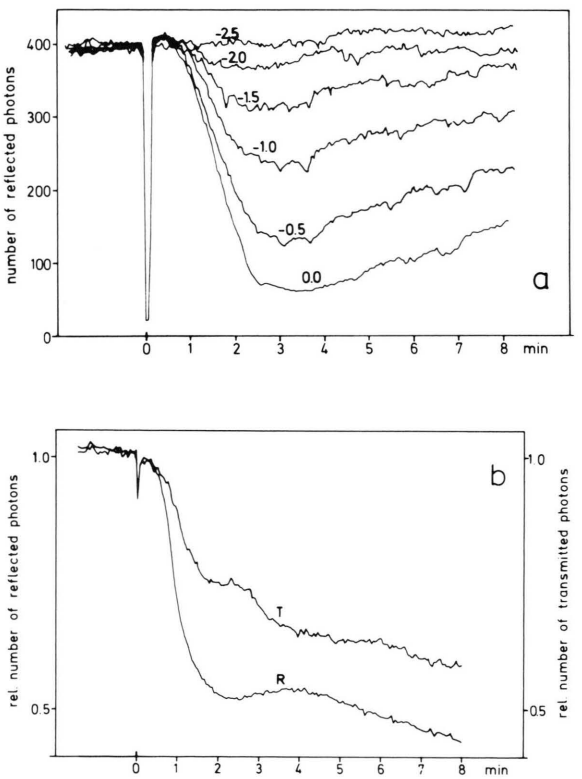

Fig. 1. Pigment migration in moth *Deilephila* elicited by blue (a) or UV (b) light stimuli, 4 s. Light stimulus indicated by artifact. a: Time course of light intensity (photons per 10 ms) reflected from eye of intact animal after stimulation by blue light (λ_{max} = 430 nm). Figure shows approximate proportionality between amplitude of decrease in reflectance and log stimulus intensity. b: Time course of change in reflectance from eye of intact animal, R, and of change in transmission through an eye preparation consisting of distal cells, T, elicited by broad band UV stimulation, 4 s. Note similar diphasic time course of R and T.

The amplitude of the decrease was approximately proportional to log stimulus intensity. A minimum was reached after about 3 min, after which the reflectance increased to reach the initial value within another 30 min. Using brighter stimuli a diphasic time course of reflectance change was generally observed (Fig. 1b, trace R). About 4 min after stimulation an incipient increase was followed by a second phase of decrease. The ensuing increase in reflectance (not seen in Fig. 1b) lasted about 2 h.

Using the same stimulating light similar time courses of transmission change were recorded from the eye preparations consisting of distal cells only (Fig. 1b, trace T). Bright stimuli were followed by a diphasic change in transmission through the eye preparation as well as in reflectance from the intact eye. It therefore seems that the complex mechanism eliciting the diphasic time course of the pigment migration is located in cells distal to the photoreceptors. This hypotheses is supported by the experiment of Fig. 2 in which noradrenaline had been added to the gel that was applied on the cornea. After the control measurement (trace 1) the animal was kept in darkness for 1 h, the gel containing noradrenaline was applied, and 10 min later the preparation was stimulated by UV light (trace 2). Unexpectedly the light stimulation did not cause the normal decrease in transmission. The opposite reaction occurred, the transmission increased, indicating that the pigment contracted instead of dispersed.

Reversed responses to light stimuli were also observed when noradrenaline, 10 mM, was applied to eye preparations consisting of distal cells only (Fig. 3). The response to a UV stimulus a few min after application of the drug was a small pigment contraction, seen as an increase in transmission (trace 2), while in the control experiment the transmission decreased showing that the pigment expanded (trace 1). About 30 min later a blue light stimulus (trace 3) elicited a large contraction, while a subsequent UV stimulus caused a smaller contraction followed by a monophasic expansion. The latter diphasic time course may be due to the high efficiency of UV light in eliciting pigment expansion [8]. A lower concentration of noradrenaline, 1 mM, also elicited a pigment contraction, although of smaller amplitude. In contrast, in the presence of adrenaline light stimulation led to a rapid decrease in transmission almost without latency (Fig. 4). Adrenaline thus enhanced the pigment expansion or possibly had no effect, *i.e.* if adrenaline had an effect it was opposite to that of noradrenaline.

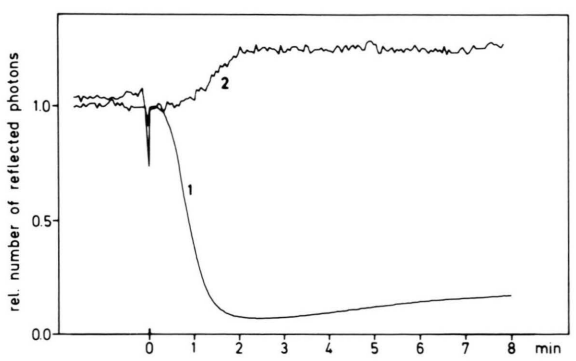

Fig. 2. Effect of noradrenaline on light-induced pigment migration. Eye of intact animal stimulated by broad band UV light, 2 s. Light stimulus indicated by artifact. Trace 1: Reflectance change in an untreated eye. Trace 2: Reflectance change in the same eye after 1 h dark adaptation and application of noradrenaline added to electrode gel. Note that in trace 2 light exposure leads to a contraction of the screening pigment instead of the normal expansion seen in trace 1.

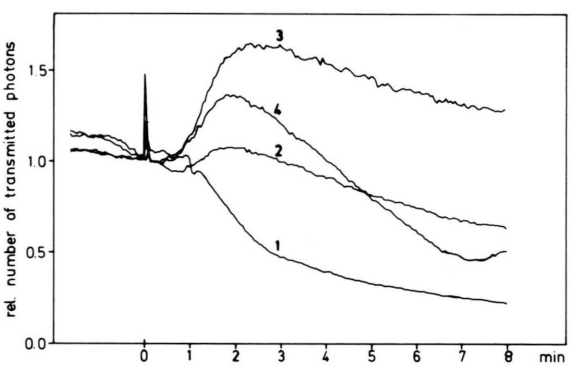

Fig. 3. Effect of noradrenaline on change in transmission through eye preparation consisting of distal cells. All measurements on same preparation. Broad band UV stimulus, 3 s (traces 1, 2 and 4) or broad band blue stimulus, 3 s (trace 2). Light stimulus indicated by artifact. Trace 1: Control experiment showing reduction in transmission through untreated eye preparation. Trace 2: Transmission change a few min after adding noradrenaline, 10 mM, to agar gel. Trace 3: Transmission change in response to blue stimulus after 30 min in darkness. Trace 4: Transmission change in response to UV stimulus after 30 min in darkness. Note that noradrenaline caused increase in transmission comparable to increase in reflectance seen in intact animal (Fig. 2).

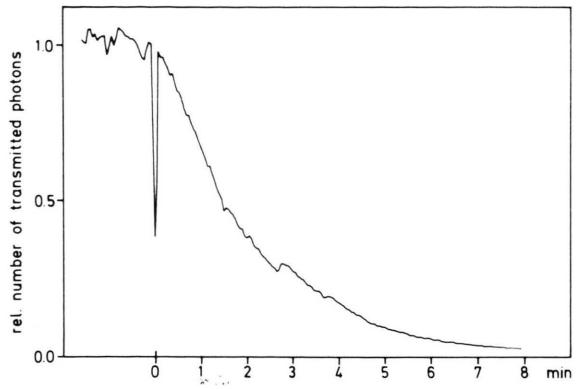

Fig. 4. Effect of adrenaline on change in transmission through eye preparation consisting of distal cells. Broad band blue stimulus, 3 s. Light stimulus indicated by artifact. Note that decrease in transmission started almost without latency, and that time course of decrease was faster than in recordings shown in Fig. 1 and 2.

Pilot experiments using dopamine showed an effect similar to that of noradrenaline, although the effect was much less pronounced, whereas phenoxybenzamine, which irreversibly blocks α-receptors [10], led to pigment expansion in darkness. This observation may mean that activation of α-receptors by noradrenaline leads to a contraction of the pigment layer. Blocking the β-receptors by dichloroisoproterenole did not change the time course of pigment expansion; a result compatible with the enhancement of expansion perhaps caused by adrenaline. Possibly related to our results is the observation (Bhatti and Fleissner, personal communication) that the application of octopamine, which also activates α-receptors, within the photoreceptors of a scorpion leads to movement of the screening pigment granules towards the dark-adapted position during the animal's light period. Our results are preliminary, but hint to a system of antagonistically acting catecholamines, possibly involved in neuro-humoral regulation of pigment migration.

Acknowledgements

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