Selective UV-induced damage to short-wavelength receptor cells in the butterfly *Papilio xuthus*

Rapid communication

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Summary. A technique that allows the specific inactivation of short-wavelength-sensitive photoreceptive cells has been needed for a long time. Such a technique could be useful in studies on the role(s) of UV-receptors in circadian rhythmicity, recognition of floral patterns, homing behaviour, and mate selection in arthropods. We provide ultrastructural evidence that short-wavelength receptor cells can be selectively damaged without affecting other spectral-cell types. Since the method does not require the killing of the experimental animal, the latter can be used in behavioural or other follow-up tests.

Keywords: Electron microscopy; UV-induced cell damage; Photoreceptor; Eye; Lepidoptera.

Introduction

For a long time researchers of photoreceptive cells in arthropods, despite the availability of visual mutants of *Drosophila melanogaster*, have been seeking ways to remove, inactive, or destroy exclusively UV-sensitive cells and none of the other types. We have found a way that achieves just that and in this brief communication we shall provide evidence that our method specifically affects the short-wavelength receptors and not the other spectral-cell types previously identified in the eye of the butterfly *Papilio xuthus* by Bandai et al. (1992). The method is based on a combination of two well known phenomena: selective adaptation (e.g., Langer et al. 1986, Rosenberg and Langer 1995) and light-induced photoreceptor damage (for a review, Meyer-Rochow 1994).

Material and methods

The experimental animal was *Papilio xuthus*; the cells examined were retinula cells from the compound eye, which occupy clearly defined positions within the optical units of the eye, i.e., the ommatidia (Fig. 1). In order to obtain fully dark-adapted material, individual butterflies were kept in total darkness for 3-5 days prior to being exposed for 3 h to UV-radiation of 350 nm wavelength and 350 µW/cm² intensity. This intensity was equivalent to about 5 times that of natural sunlight and 50 times that of a blue sky over Yokohama City. Since it was known from previous studies on the crayfish eye (Meyer-Rochow and Eguchi 1983) that UV-induced damage manifested itself clearly not until 24 h after the exposure, irradiated butterflies were given a 24-h recuperation period in the dark before their eyes were fixed for electron microscopic observations. To test whether the damage was accumulative, some of the butterflies, following the one-day recuperation period, were not immediately fixed, but exposed to the same UV-light source over the same duration for a second and even a third time.

In order to guarantee that the exposed eyes received the full amount of radiation, animals were fixed with tape in such a way that the right compound eyes faced the UV-light source. To indicate the correct dorso-ventral orientation of an eye after its removal from the butterfly head in dim red light, the central region of the eye was dissected out in the form of a trapezoid block. Fixation, postfixation, dehydration, and embedding procedures were standard and have been described elsewhere. Once embedded, the tissues were trimmed and thin-sectioned, but because of their trapezoid shapes, the dorso-ven-
Results and discussion

About 30–40 ommatidia of each compound eye (n = 6) were examined for signs of damage in the distal, most vulnerable region of the rhabdom and about half of these displayed obvious signs of UV-induced damage when compared with nonirradiated controls (Fig. 2). The damage involved almost always the receptor pairs R1 and R2 (cell nomenclature follows Ribi 1987) representing UV-, violet-, or blue (= short-wavelength)-sensitive cells and was accumulative, i.e., strongest in the repeatedly irradiated specimens (Fig. 3). Out of 146 ommatidia from eyes that had been irradiated three times, those with all eight retinula cells apparently undamaged amounted to 35 (24%); in 97 (66%) of all ommatidia, however, both R1 and R2 were damaged while the remainder (R3–R8) were normal; the dorsally-positioned R1 alone was damaged in 9 ommatidia (6%), while the ventrally-pointing R2 on its own displayed damage in only 5 (2%) of all ommatidia examined. In conclusion, signs of photo-induced stress occurred predominantly in the short-wavelength receptor cells R1 and R2 and almost always affected the two cells together. In cases where only one cell of the pair showed damage, it was more frequently the dorsally-positioned one.

Maximum damage manifested itself by the following symptoms to cells R1 and R2 (cf. Fig. 2, depicting normal cells, with Fig. 3 showing UV-damage): (1) shrinkage of cell volume and nucleus, (2) increased electron opacity of cytoplasm and nucleus, (3) greater density and compactness of nucleus (cf. Fig. 4 of normal nucleus with corresponding UV-injured one in Fig. 5), (4) swelling of mitochondria and ruptures of their internal membranes (Fig. 6), (5) elevated number of lysosomal elements and multivesicular bodies (Fig. 6). More generally, and not necessarily restricted to cells R1 and R2, the distal layers of the irradiated rhabdoms contained significantly more small electron-dense particles than the nonirradiated controls (Fig. 7). Tubular, intracellular membrane specializations, known as “trophospongium-like structures” and thought to be involved in the supply or removal of substances during periods of stress (Eguchi and Meyer-Rochow 1983), occurred in the cytoplasm of the damaged as well as other cells of the irradiated eyes (Fig. 8). The rhabdom itself, in view of the damage it suffers in the irradiated eyes of crustacea (cf. review by Meyer-Rochow 1994), displayed a surprisingly coherent organization and the 9 axons leading from the ommatidium (including that of the basal cell R9) showed no apparent signs of damage at the time of fixation (Fig. 9). This agrees with postmortem observations on fly photoreceptor cells, in which signs of cell death were found to proceed from the periphery inward toward the centre, affecting rhabdomeres last (Meyer-Rochow and Järvilehto 1997b), and with findings from the crab compound eye in which light-induced changes in microvillar diameters were most pronounced distally (Meyer-Rochow and Reid 1994). Solar-damaged rods in a vertebrate (the albino rainbow trout), however, exhibited a very different pattern and showed that cell bodies can persist on their own without the photoreceptive membranes of the outer segments (Allen and Hallows 1997).

In summary, the method outlined and tested above, seems to selectively affect the short-wavelength receptor cells, for they were the ones that showed the greatest cytoplasmic effects following exposure to UV-radiation. Since stimulation of the photoreceptive cells, generally, activates the aerobic metabolism in