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Short Communication

Ultraviolet Radiation Induces Dose-Dependent Pigment Dispersion in Crustacean Chromatophores

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Pigment dispersion in chromatophores as a response to UV radiation was investigated in two species of crustaceans, the crab *Chasmagnathus granulata* and the shrimp *Palaemonetes argentinus*. Eyestalkless crabs and shrimps maintained on either a black or a white background were irradiated with different UV bands. In eyestalkless crabs the significant minimal effective dose inducing pigment dispersion was 0.42 J/cm^2 for UVA and 2.15 J/cm^2 for UVB. Maximal response was achieved with 10.0 J/cm^2 UVA and 8.6 J/cm^2 UVB. UVA was more effective than UVB in inducing pigment dispersion. Soon after UV exposure, melanophores once again reached the initial stage of pigment aggregation after 45 min. Aggregated erythrophores of shrimps adapted to a white background showed significant pigment dispersion with 2.5 J/cm^2 UVA and 0.29 J/cm^2 UVC. Dispersed erythrophores of shrimps adapted to a black background did not show any significant response to UVA, UVB or UVC radiation. UVB did not induce any significant pigment dispersion in shrimps adapted to either a white or a black background. As opposed to the tanning response, which only protects against future UV exposure, the pigment dispersion response could be an important agent protecting against the harmful effects of UV radiation exposure.

Keywords: Ultraviolet radiation, Melanophore, Erythrophore, Crab, Shrimp

INTRODUCTION

Several studies have demonstrated a relationship between increased pigmentation in mammals and UV radiation (1–4). In addition, some studies have verified the tanning effect induced by UV radiation in other groups of vertebrates. As examples, juvenile hammerhead sharks showed an increase of melanin content in the skin as a direct response to UV (5), and embryos of the spotted salamander also increased melanin production in response to UV radiation (6).

Translocation of pigment granules within pigment cells (chromatophores) in response to UV has been observed in some species of fish and amphibians. Melanophores from larvae of two fish species, *Engraulis mordax* and *Scomber japonicus*, showed pigment dispersion in response to UVA and UVB (7). However, melanophores of tadpoles and fish showed pigment aggregation after UVC exposure (8–9).

In crustaceans, only a few studies have been performed to verify this response. Melanophores of the fiddler crab *Uca pugilator* showed immediate pigment dispersion in response to near UV (300–400 nm) radiation obtained with a black-light lamp. However, this response was not observed with far UV (200–300 nm) radiation, obtained with a germicide lamp (10).

Organisms inhabiting oligotrophic waters, water surface or shallow inshore waters are often exposed to high UV intensities. With this background in mind, the objective of the present study was to compare the pigment dispersion response after exposure to different bands of UV radiation in two species of crustaceans, the intertidal crab *Chasmagnathus granulata* and the shrimp *Palaemonetes argentinus*.

Abbreviations – D, dark; L, light

MATERIAL AND METHODS

Animal Capture and Acclimation

Adult male crabs (*C. granulata*) were captured in salt marshes near the city of Rio Grande (Southern Brazil). In the laboratory, crabs were acclimated to salt water at 20‰ salinity, for at least 7 d. During acclimation, they were fed ground beef three times a week '*ad libitum*'. Shrimps (*P. argentinus*) were captured in the Lagoa dos Patos estuary (Rio Grande, Brazil), transferred to the laboratory and kept in aquaria containing water at 10‰ salinity. They were acclimated for at least 7 d and were fed commercial rations (Kijaro Grow, Malasia) '*ad libitum*' three times a week. For both species room temperature and photoperiod were fixed at 20°C and 12 h light (L):12 h dark (D), respectively.

Animal Models

As intact *C. granulata* with both eyestalks did not show pigment aggregation in response to a background color change, eyestalkless crabs were used to obtain animals with melanophores in an aggregated state before UV exposition. The eyestalk ablation was performed 24 h before UV exposure (11).

The shrimp *P. argentinus* was employed to verify if a different species, with a different type of pigment cells (erythrophores), shows a similar response to that displayed by crabs. In preliminary experiments, this shrimp species showed a fast and intense physiological color change in response to background color. Shrimps adapted to a white background remained with aggregated erythrophores, while those adapted to a black background remained with these same cells dispersed.

UV Exposure

Both crustaceans, eyestalkless crabs and shrimps ($n = 10$), maintained on a black or a white background, were irradiated with UVA (VL: 115 L, 30 W) and UVB (VL: 115 C, 30 W; Vilber Lourmat, Marne Lavalee, France) lamps. Only shrimps were irradiated with a UVC lamp (VL: 115 C, 30 W). UVA and UVB irradiation were monitored using a radiometer/photometer (model IL 1400A, International Light, Newburyport, MA, USA). UVC irradiation was monitored using a radiometer/ultraviolet meter (model J-225, Blak-Ray Inc., San Gabriel, CA, USA). The UVA lamp produced 1.39 mW/cm^2 UVA and 0.006 mW/cm^2 UVB, with contamination of 928.0 nW/cm^2 visible light. The UVB lamp produced 493 μW/cm^2 UVA and 1.195 mW/cm^2 UVB, with contamination of 0.113 μW/cm^2 visible light. Neither lamp showed contamination with UVC. The UVC lamp produced 0.3 mW/cm^2 UVC with contamination of 35.9 μW/cm^2 UVA, 0.052 mW/cm^2 UVB and 0.075 μW/cm^2 visible light.

Control groups were maintained under fluorescent lamps (Philips TLT 40 W/75, Sâo Paulo, Brazil) irradiating 96.0 mW/cm^2 visible light. Different doses of UV (0.08, 0.42, 1.25, 2.5, 5.0 and 10.0 J/cm^2 UVA; 0.07, 0.36, 1.07, 2.15, 4.3 and 8.6 J/cm^2 UVB and 0.09, 0.29 J/cm^2 UVC) were obtained using different exposure times. During UV exposure, animals were maintained under the same conditions used for acclimation, but without feeding.

Pigment Dispersion Determination

Pigment dispersion was quantified using an index which establishes stage 1 as full pigment aggregation, stage 5 as full dispersion, and stages 2, 3 and 4 as intermediary conditions (12). Pigment dispersion was analyzed in melanophores from the meropodit of the third pair of maxilipeds and erythrophores in the dorsal region of the abdomen, in crabs and shrimps, respectively. The degree of pigment dispersion was measured before and immediately after UV exposure. To obtain a dose–response curve the difference between these two values was calculated.

Protection Against UV Effects by the Carapace

To verify the amount of UV actually reaching the tegument, crab and shrimp exoskeletons were removed and washed with tap water to remove any adherent tissue or dust. Pools of 18 shrimp exoskeletons and two crab exoskeletons were used to completely cover the photocell unit of the radiometer. UV irradiation passing through the exoskeleton was then measured and compared with the total incident UV.

Statistical Analysis

To verify differences in pigment dispersion as a function of UV exposure, data were subjected to one-way variance analysis followed by the Student–Newman Keuls test. The dose–response curve was obtained by using a non-linear regression model. The significance level adopted was 95%. All statistical analysis was performed using the software STATISTICA 5.1 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

Regarding the capacity of the exoskeleton to block UV radiation, there was a significant difference between crabs and shrimps. In the crab, the dorsal exoskeleton blocked 94.9% ± 1.7 UVA, 96.4% ± 1.3 UVB and 100% UVC radiation. In the shrimp, the dorso-lateral exoskeleton blocked only 25% UVA, 42.5% UVB and 52.5% UVC radiation.

UVA and UVB radiation induced significant dose-dependent pigment dispersion in crab melanophores (Fig. 1). The significant minimal effective dose was 0.42 J/cm^2 for UVA and 2.15 J/cm^2 for UVB, and maximal response was achieved at 10.0 J/cm^2 UVA and 8.6 J/cm^2 UVB. UVA was more effective than UVB to induce pigment dispersion. Soon after UV exposure, melanophores showed pigment aggregation, reaching the initial stage after 45 min (data not shown).

Shrimps adapted to a white background, i.e. with aggregated erythrophores, showed significant pigment dispersion with 2.5 J/cm^2 UVA and 0.29 J/cm^2 UVC. Shrimps adapted to a black background, i.e. with dispersed erythrophores, did not show any significant response to UVA or UVC radiation. UVB did not induce significant pigment dispersion in

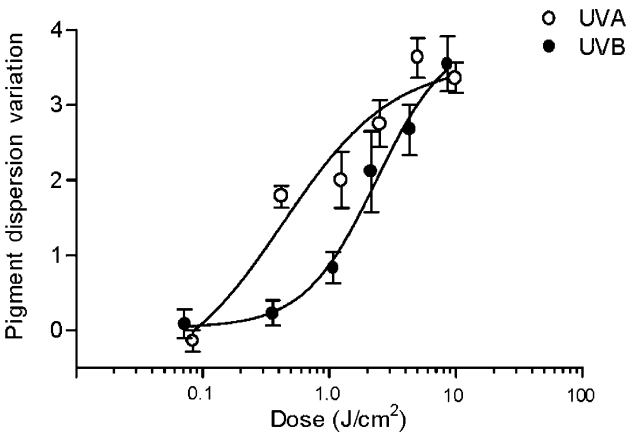

Fig. 1. Dose–response curves of the degree of pigment dispersion in melanophores of eyestalkless *Chasmagnathus granulata* subjected to UVA and UVB exposure. Values were obtained through the difference between values before and after UV exposure. Data expressed as mean ± standard error of mean (n = 10).

shrimps adapted to either a white or a black background (Fig. 2).

DISCUSSION

As already noted, melanophores of eyestalkless *U. pugilator* exposed to near UV (300–400 nm) radiation, obtained with a blacklight lamp, showed immediate and dose-dependent pigment dispersion. However, this response was not observed with far UV (200–300 nm) radiation obtained with a germicide lamp (10). When intact crabs were also exposed to near UV (300–400 nm) radiation during the night-phase of their circadian rhythm, i.e. when they have their black pigments aggregated, a similar pigment response was reported (13). Studies with larvae and postlarvae of crab species also demonstrated that sunlight lamps (292–400 nm) induce black pigment dispersion (14–15). In the present study, specific lamps for UVA, UVB or UVC were employed. UVC was utilized only in the shrimp *P. argentinus* because the crab exoskeleton completely blocks this radiation.

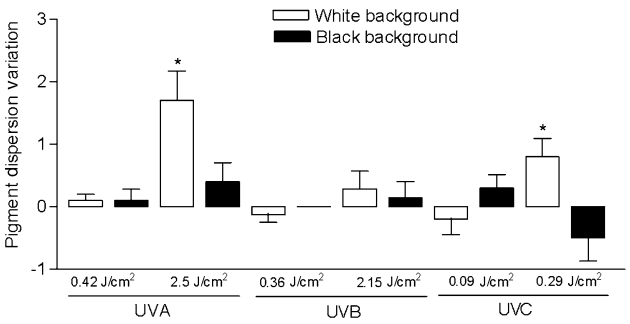

Fig. 2. Variation in pigment dispersion in erythrophores of *Palaemonetes argentinus* adapted to a black or a white background, irradiated with different doses of UVA (0.42 and 2.5 J/cm^2), UVB (0.36 and 2.15 J/cm^2) or UVC (0.09 and 0.29 J/cm^2). Values were obtained through the difference between values before and after UV exposure. Data expressed as mean ± standard error of mean (n = 10). *Indicates mean significantly different from the control ($P < 0.05$).

UVA radiation induced dose-dependent pigment dispersion in melanophores of eyestalkless crabs and pigment dispersion in shrimp erythrophores. UVB radiation also induced dose-dependent pigment dispersion in melanophores of eyestalkless crabs. It is also important to note that this pigment dispersion was observed in the absence of neurohormones from the major neuroendocrine system located in the optical ganglia of the eyestalk (X Organ–Sinus Gland Complex). Also, the melanophores return to pigment aggregation soon after UV exposure, suggesting that the process of pigment dispersion by UV radiation is not due to cell damage, but is a process of physiological color change.

It is known that the delayed tanning response in mammals can be induced by both UVA and UVB radiation, although the response induced by UVA alone is two to three orders of magnitude less efficient (16). In the present study, UVB radiation was less effective than UVA radiation in inducing pigment dispersion. In addition, UVB radiation up to 2.15 J/cm^2 was not able to induce pigment dispersion in shrimp erythrophores. Perhaps larger doses of UVB could induce such dispersion.

Despite the fact that UVC does not reach the Earth surface, some studies have analyzed and compared the effects of UVC and UVB radiation on the main cell target, i.e. the DNA molecule. These studies have demonstrated that UVC radiation increased the effects of UVB radiation. In amphibians and fish melanophores, UVC induced pigment aggregation (8, 9). Preliminary results obtained in our laboratory, employing a germicide lamp, also demonstrated pigment aggregation in erythrophores of shrimps adapted to black background (data not shown). However, employing a specific lamp for UVC radiation we observed significant pigment dispersion in erythrophores of *P. argentinus* adapted to a white background. On the other hand, when these animals were adapted to a black background and irradiated with UVC, they presented a tendency towards pigment aggregation. Perhaps higher doses of UVC are necessary to generate a full aggregation response.

In conclusion, our results show that crustaceans exposed to either UVA, UVB or UVC respond with a rapid pigment dispersion inside chromatophores. In contrast to the tanning response, which only protects against future UV exposure, this pigment migration could be an important response protecting against harmful effects of UV radiation during its application.

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