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Effect of Cadmium Chloride on the Distal Retinal Pigment Cells of the Fiddler Crab, *Uca pugilator*

P. S. Reddy,[1] L. K. Nguyen,[2] P. 0bih,[2] M. Fingerman[1]

[1]Department of Ecology, Evolution, and Organismal Biology, Tulane University, New Orleans, Louisiana 70118, USA
[2]College of Pharmacy, Xavier University, New Orleans, Louisiana 70125, USA

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Crustaceans have two sets of pigmentary effectors, chromatophores and retinal pigment cells. Chromatophores, located mainly in the integument, enable the organism to change color. Retinal pigments (distal, proximal and reflecting) control the amount of light striking the rhabdom, the photosensitive portion of each ommatidium, screening the rhabdom in bright light and uncovering it in darkness or dim light.

Migration of the distal pigment in the fiddler crab, *Uca pugilator*, is regulated by a light-adapting hormone and a dark-adapting hormone (Fielder et al. 1971). Likewise, the black chromatophores of this crab are also controlled by a pair of hormones, the antagonistically acting black pigment-dispersing hormone (Carlson 1935) and black pigment-concentrating hormone (Fingerman 1956). Both pigmentary effectors exhibit circadian rhythms. Even when *Uca pugilator* is maintained in constant darkness the pigment in its black chromatophores becomes more dispersed by day than at night (Abramowitz 1937). Likewise, even in constant darkness the distal pigment of this crab approaches the fully light adapted position by day and then goes to the fully dark adapted position at night (Fingerman 1970).

The effects of some organic and inorganic pollutants on the ability of *Uca pugilator* to change color have been described. Exposure of this crab to naphthalene (Staub and Fingerman 1984) or cadmium (Reddy and Fingerman 1995) results in decreased ability to disperse the pigment in their black chromatophores, the exposed crabs becoming paler than the unexposed crabs. Norepinephrine triggers release of both the black pigment-dispersing hormone (Fingerman et al. 1981) and the light-adapting hormone (Kulkarni and Fingerman 1986). In view of the facts that (a) these hormones which regulate the black chromatophores and distal pigment are synthesized in and released from the eyestalk neuroendocrine complex (Kulkarni and Fingerman 1991), (b) the black pigment-dispersing hormone and the light-adapting hormone may actually be the same hormone, having two different activities (Riehm and Rao 1982) and (c)

Correspondence to: M. Fingerman

release of both the black pigment-dispersing hormone and the light-adapting hormone is triggered by norepinephrine, the present investigation was carried out to determine the effect of cadmium on distal pigment migration in *Uca pugilator*. More specifically, for comparison with the previously reported effect of cadmium on pigment migration in the black chromatophores (Reddy and Fingerman 1995), we wished to determine whether the distal pigment of fiddler crabs exposed to cadmium chloride is capable of as wide a range of movement as in unexposed crabs, and if not what might be the explanation. This is the first report of the effect of a pollutant on a retinal pigment of any crustacean.

MATERIALS AND METHODS

Fiddler crabs, verified as *Uca pugilutor*, were obtained from the Gulf Specimen Co. (Panacea, FL). The stock supply of crabs was maintained in a recirculating seawater system under a 12:12 L:D regime, lights on at 0800, and at 24° C. The illumination in the stock tank was 450 lx. Adult female crabs with a carapace width of 15-18 mm were selected from the stock for use 24 hr prior to the start of each experiment. In all experiments the crabs were put into round (18 cm id), black enameled pans that contained 500 mL seawater. The seawater (clean or cadmium-containing) in all the pans was changed every day in an effort to maintain a constant concentration of cadmium in the appropriate container as well as to minimize accumulation of excretory products. The crabs were not fed during an experiment.

The position of the distal pigment was determined by using the method of Fingerman (1970). Briefly, each crab was immersed, ventral surface down, in a clear glass dish of seawater on the stage of a stereoscopic dissecting microscope. When the crab extended an eyestalk, two measurements were made with the aid of an ocular micrometer and transmitted light: (1) the width of the widest portion of the translucent portion of the eye and (2) the width of the entire eye stalk along the line where the first measurement was made These two measurements required only a few seconds of illumination. This brief illumination had no noticeable effect on the position of the distal pigment. The ratio of the width of the translucent portion divided by the total width is called the distal pigment index (DPI). In bright light the translucent portion is widest as the distal pigment migrates proximally to shield the rhabdom, and narrows in darkness or dim light as the distal pigment migrates distally to uncover the rhabdom. Because of the circadian rhythm of migration of the distal pigment, all measurements were made between 1000 and 1100 hr. The data were analyzed by means of Student's t-test with significance set at the 95% confidence interval. Standard errors of the means were also calculated.

For use in the initial set of experiments two groups of 20 crabs each, taken from the stock supply, were placed into pans that contained clean seawater and then put into a darkroom where they were kept for the entire length of the experiment

Table 1. Effect of cadmium chloride on the distal pigment index (DPI) of crabs maintained in darkness. Mean ± SEM, N = 40.

	Distal Pigment Index (DPI)	
Days	Cadmium-exposed	Control (no cadmium)
0	0.114 ± 0.007	0.117 ± 0.007
1	0.088 ± 0.006	0.114 ± 0.006
2	0.087 ± 0.006	0.109 ± 0.005
3	0.086 ± 0.007	0.112 ± 0.006
6	0.092 ± 0.008	0.117 ± 0.007

Twenty-four hr after the crabs had been put into the darkroom one group was exposed to 10 mg/L cadmium chloride and the second group was kept in clean seawater to serve as the control group. This is the same concentration of cadmium chloride used by Reddy and Fingerman (1995) in their study of the effect of cadmium on the black chromatophores of *Uca pugilator*. The DPI of all 40 crabs were determined at the time the experimental group was first put into the cadmium-containing seawater (day 0) and after 1, 2, 3 and 6 d of exposure. The experiment was repeated once with consistent results.

For use in the second set of experiments, crabs were selected from the stock and put into the darkroom in black enameled pans for 24 hr prior to the start of the experiment. After 24 hr 40 crabs were selected and were divided into two groups of 20 crabs each. One group was exposed to 10 mg/L cadmium chloride and the other served as the control. After 6 d both groups were taken from the darkroom and exposed to a bright light (2100 lx). The DPI of all the crabs was determined at the time the crabs were removed from the darkroom and after 2, 4 and 6 hr. This experiment was also repeated one time.

For use in the final set of experiments two groups of 10 crabs each that had been exposed for 6 d to 10 mg/L cadmium chloride and two groups of control crabs, 10 in each group, all kept in the darkroom throughout the experiment, were prepared. The crabs in one cadmium-exposed group and one control group were each injected with 50 µL crab saline (Cooke et al, 1977). The remaining cadmium-exposed crabs and control crabs each received 20 µg of norepinephrine dissolved in 50 µL of saline. Throughout this experiment the crabs remained in the darkroom. The DPI of all the crabs were determined 1 and 2 hr after injection, This experiment was also done twice.

Table 2. Responses of the distal pigment to bright illumination (2100 lx) after 6 d of exposure to cadmium chloride in a darkroom. Mean ± SEM, N = 40.

	Distal Pigment Index (DPI)	
Hours	Cadmium-exposed	Control (no cadmium)
0	0.088 ± 0.006	0.117 ± 0.006
2	0.103 ± 0.005	0.133 ± 0.004
4	0.103 ± 0.006	0.135 ± 0.003
6	0.107 ± 0.005	0.133 ± 0.003

RESULTS AND DISCUSSION

The aim of the initial set of experiments was to determine whether cadmium exposure affects the DPI of crabs kept in a darkroom. The averaged results of this initial set of experiments are presented in Table 1. The mean DPI of the cadmium-exposed crabs was not significantly different from the DPI of the unexposed crabs at the start of the exposure (day 0). However the DPI of the cadmium-exposed crabs were significantly smaller than the corresponding DPI of the control crabs on days 1 ($P < 0.01$), 2 ($P < 0.01$), 3 ($P < 0.01$) and 6 ($P < 0.05$). None of the differences among the control DPI were significant. With respect to the data for the cadmium-exposed crabs, the DPI for days 1, 2, 3 and 6 showed no significant differences among themselves. However, the DPI of the cadmium-exposed crabs on day 0 was significantly larger than the DPI of the exposed crabs on days 1 ($P < 0.01$), 2 ($P < 0.01$), 3 ($P < 0.01$) and 6 ($P < 0.05$).

The aim of the second set of experiments was to determine whether the distal pigment of crabs kept in the darkroom during 6 d of exposure to cadmium could attain the same light adaptational level upon being subjected to bright illumination as could the distal pigment of crabs not exposed to cadmium. The averaged results of the second set of experiments are presented in Table 2. The mean DPI of the cadmium-exposed crabs was significantly less than that of the control crabs after the 6 d of cadmium exposure, i.e., at the time the crabs were about to be exposed to the bright illumination ($P < 0.01$ at 0 hr). The distal pigment of the cadmium-exposed crabs did not attain the same level of light adaptation in response to the bright illumination as did the distal pigment of the unexposed crabs. Although the distal pigment of the cadmium-exposed crabs was able to migrate toward the fully light adapted position, the mean DPI of the control crabs were significantly larger than the mean DPI of the cadmium-exposed crabs throughout the 6 hr exposure to bright illumination ($P < 0.001$ after 2, 4 and 6 hr). With respect to the cadmium-exposed crabs alone, the differences between the 0 hr

Table 3. Response of the distal pigment of cadmium-exposed and unexposed (control) crabs to norepinephrine (NE) and saline. Mean ± SEM, N = 20.

	Distal Pigment Index (DPI)			
	Cadmium-exposed		Control (no cadmium)	
Hours	NE	Saline	NE	Saline
1	0.116 ± 0.008	0.095 ± 0.007	0.134 ± 0.002	0.117 ± 0.007
2	0.113 ± 0.007	0.090 ± 0.005	0.142 ± 0.004	0.128 ± 0.010

DPI and the 2 and 4 hr DPI were not significant, but the difference between the 0 hr and 6 hr readings was significant ($P < 0.02$). In regard to the control values alone, the differences between the 0 hr DPI and the 2, 4 and 6 hr DPI were significant, $P < 0.05$, 0.02 and 0.05, respectively.

The final set of experiments was conducted to determine the responses of the distal pigment to norepinephrine and physiological saline of cadmium-exposed and unexposed (control) crabs. As stated above, norepinephrine triggers release of the light-adapting hormone. As in the previous experiments, the mean DPI of all the cadmium-exposed groups of crabs were smaller than those of the corresponding unexposed groups (Table 3). But norepinephrine did induce migration of the distal pigment in both the unexposed and cadmium-exposed crabs toward the fully light adapted position. Specifically, with respect to the cadmium-exposed crabs, the differences between the DPI of the crabs administered norepinephrine and the DPI of the crabs administered saline were significant after both 1 and 2 hr, $P < 0.05$ and $P < 0.02$, respectively. With regard to the control crabs, the differences between the DPI of the crabs administered norepinephrine and the DPI of the crabs administered saline also were significant after 1 and 2 hr, $P < 0.05$ for both. Likewise, the differences between the DPI of the cadmium-exposed crabs administered saline and the DPI of the control crabs administered saline were significant after 1 and 2 hr, $P < 0.05$ and $P < 0.02$, respectively. Furthermore, the differences between the DPI of the cadmium-exposed crabs administered norepinephrine and the DPI of the control crabs administered norepinephrine were significant after 1 and 2 hr, $P < 0.05$ and $P < 0.01$, respectively.

Exposure to cadmium chloride resulted in decreased ability of the distal pigment to migrate toward the fully light adapted position. The distal pigment of the cadmium-exposed crabs did not attain as large a DPI as did the crabs kept in clean water whether simply kept in the darkroom (Table 1) where because of the circadian rhythm the distal pigment would migrate toward the fully light adapted

position or when exposed to bright illumination (Table 2) after 6 d of cadmium exposure. These decreased abilities have survival implications. Decreased ability to control the amount of light striking the rhabdom can certainly affect the ability to survive in the wild. As stated above, exposure to naphthalene or to cadmium resulted in decreased pigment dispersion in the black chromatophores of *Uca pugilator* (Staub and Fingerman 1984; Reddy and Fingerman 1995). Staub and Fingerman (1984) provided evidence to support the hypothesis that the inhibition of black pigment dispersion produced by exposure to naphthalene was due to inhibition of norepinephrine release. Perhaps cadmium similarly inhibits norepinephrine release, which because norepinephrine triggers release of both the black pigment-dispersing hormone and the light-adapting hormone (Fingerman et al. 1981; Kulkarni and Fingerman 1986), would result in reduced black pigment-dispersing hormone and light-adapting hormone release and hence less dispersion of the pigment in the black chromatophores and less light adaptation of the distal pigment. Norepinephrine was able to produce light-adapting responses in the cadmium exposed crabs (Table 3) which offset at least in part the inhibitory effect of cadmium on the distal pigment. Future study of the effects of pollutants on these two pigmentary effectors, chromatophores and retinal pigments, should provide additional interesting comparative data. These pigment cells may prove to be important biomarkers.

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REFERENCES

Abramowitz AA (1937) The chromatophorotropic hormone of the Crustacea: Standardization, properties and physiology of the eye-stalk glands. Biol Bull 72: 344-365

Carlson SP (1935) The color changes in *Uca pugilator*. Proc Natl Acad Sci USA 21:549-551

Cooke IM, Haylett BA, Weathersby TM (1977) Electrically elicited neurosecretory and electrical responses of the isolated crab sinus gland in normal and reduced calcium salines. J Exp Biol 70: 125-149

Fielder DR, Rao KR, Fingerman M (1971) Control of distal retinal pigment migration in the fiddler crab *Uca pugilator*. Mar Biol 9:219-223

Fingerman M (1956) Black pigment concentrating factor in the fiddler crab. Science 123:585-586

Fingerman M (1970) Circadian rhythm of distal retinal pigment migration in the fiddler crab, *Uca pugilator*, maintained in constant darkness and its endocrine control. J Interdiscipl Cycle Res 1: 115-121

Fingerman M, Hanumante MM, Fingerman SW, Reinschmidt DC (1981) Effects of norepinephrine and norepinephrine agonists and antagonists on the melanophores of the fiddler crab *Uca pugilator*. J Crust Biol 1: 16-27

Kulkarni GK, Fingerman M (1986) Distal retinal pigment of the fiddler crab, *Uca pugilator:* Evidence for stimulation of release of light adapting and dark adapting hormones by neurotransmitters Comp Biochem Physiol 84C: 219-224

Kulkarni GK, Fingerman M (1991) Release of black pigment-dispersing hormone and distal retinal pigment light-adapting hormone upon electrical stimulation of the isolated eyestalk neuroendocrine complex of the fiddler crab, *Uca pugilator.* Comp Biochem Physiol 99C: 47-52

Reddy PS, Fingerman M (1995) Effect of cadmium chloride on physiological color changes of the fiddler crab, *Uca pugilator.* Ecotoxicol Environ Safety 31: 69-75

Riehm JP, Rao KR (1982) Structure-activity relationships of a pigment-dispersing crustacean neurohormone. Peptides 3:643-647

Staub GC, Fingerman M (1984) A mechanism of action for the inhibition of black pigment dispersion in the fiddler crab, *Uca pugilator ,* by naphthalene. Comp Biochem Physiol 79C: 447-453