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Cadmium and Naphthalene-Induced Hyperglycemia in the Fiddler Crab, *Uca pugilator:* Differential Modes of Action on the Neuroendocrine System

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Hyperglycemia is a typical sublethal response of aquatic organisms to heavy metals (Haux et al. 1986). In crustaceans, the medulla terminalis X-organ-sinus gland neuroendocrine complex in the eyestalk is the source of the crustacean hyperglycemic hormone (CHH) . The role of CHH in pollutant-induced blood glucose changes has only recently begun to be studied. Reddy et al (1994) provided evidence that CHH mediates cadmium-induced hyperglycemia in the red swamp crayfish, *Procambarus clarkii.* In a study of another hormonally-regulated function, color changes, cadmium exposure resulted in pigment in the melanophores of the fiddler crab, *Uca pugilator,* becoming less dispersed than in unexposed crabs (Reddy and Fingerman 1995). Earlier studies (Fingerman and Fingerman 1978; Staub and Fingerman 1984) showed that, like cadmium, both a PCB, Aroclor 1242, and naphthalene induced black pigment aggregation in *Uca pugilator.* In general, when crabs are exposed to a pollutant, hydrocarbon or cadmium, they aggregate the pigment in their melanophores, but apparently by different mechanisms. Hydrocarbons appear to inhibit release of black pigment-dispersing hormone (BDPH), whereas cadmium appears to inhibit its synthesis. These apparent different modes of action of cadmium and naphthalene on the color change mechanism led us to compare the impact of these pollutants on the hormonal regulation of blood glucose in *Uca pugilator.*

The present study was performed to determine (1) whether cadmium and naphthalene induce hyperglycemia in *Uca pugilator,* (2) whether CHH has a role, if naphthalene and cadmium do induce hyperglycemia, and (3) the effects, if any, of cadmium and naphthalene on CHH activity in the eyestalk neuroendocrine complex.

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MATERIALS AND METHODS

Fiddler crabs, verified as *Uca pugilator,* were used. They were obtained from the Gulf Specimen Co., Panacea, FL, and were acclimated to the laboratory conditions, 23-25°C, for a week before use. Twenty-four hours prior to the start of each experiment, male intermolt crabs having a carapace width of 13-15 mm were selected from the stock for use. These crabs were kept in round (20-cm diameter) glass containers covered with glass plates, 10 crabs in each container, that contained 500 ml of seawater 15 ppt. The water (clean seawater and seawater containing cadmium chloride or naphthalene) in each bowl was changed every day in an effort to maintain near constant concentrations of cadmium and naphthalene in the appropriate containers as well as to minimize the buildup of excretory products. The crabs were not fed during an experiment.

Experiment I used four groups of crabs.
(1A) Intact crabs in clean water
(1B) Intact crabs exposed to 5 ppm cadmium chloride
(1C) Eyestalkless crabs in clean water
(1D) Eyestalkless crabs exposed to 5 ppm cadmium chloride

Experiment II used four groups of crabs.
(2A) Intact crabs in clean water
(2B) Intact crabs exposed to 5 ppm naphthalene
(2C) Eyestalkless crabs in clean water
(2D) Eyestalkless crabs exposed to 5 ppm naphthalene

Experiment III used seven groups of crabs.
(3A) Intact crabs in clean water
(3B) Intact crabs exposed to 5 ppm cadmium chloride for 10 days
(3C) Intact crabs exposed to 5 ppm naphthalene for 10 days
(3D) Intact crabs injected with crab physiological saline
(3E) Intact crabs injected with an extract prepared from the eyestalks of Group 3A
(3F) Intact crabs injected with an extract prepared from the eyestalks of Group 3B
(3G) Intact crabs injected with an extract prepared from the eyestalks of Group 3C

Eyestalk extracts were prepared by homogenizing the eyestalk tissue in crab physiological saline (Cooke et al. 1977) and then centrifuging at 10,000 g for 10 min at 4°C. The supernate was used for injections. Each crab received 0.5 eyestalk equivalent in a 50 µl injection. Blood was drawn 2 hr after injection and glucose levels were determined in the following manner. Fifty µl of blood were deproteinized using 150 µl of 6% perchloric acid and then centrifuged. The supernate was used for glucose determination using the Sigma glucose oxidase

Table 1. Blood glucose levels (mg glucose per 100 ml blood) of intact eyestalk-ablated crabs, *Uca pugilator.* See text for key to groups. Values are given as mean ± SEM. N=20. *: P<0.001, versus control.

	Exposure period (Days)				
	2	4	6	8	10
Group 1A	10.44 ± 0.44	11.66 ± 0.72	10.44 ± 0.66	10.50 ± 0.82	11.74 ± 0.88
Group 1B	11.38 ± 0.66	12.30 ± 0.74	15.04 ± 0.68*	16.78 ± 1.50*	21.22 ± 1.44*
Group 1C	11.06 ± 0.82	12.06 ± 0.60	11.50 ± 0.92	11.32 ± 0.66	11.80 ± 0.74
Group 1D	11.24 ± 0.80	11.20 ± 0.70	11.92 ± 0.76	11.88 ± 0.76	12.14 ± 0.64

diagnostic kit (Procedure No. 510) (Sigma Chemical Co., St Louis, MO). Each experiment was repeated once. For each group the means of the values determined are presented with the SEM. The data were analyzed also by use of Student's t test to compare means for statistical significance, and a $P<0.05$ was interpreted as being significant.

RESULTS AND DISCUSSION

The blood glucose concentration in the cadmium-exposed intact fiddler crabs (Group 1B) increased significantly compared to the crabs in cadmium-free water (Group 1A) (Table 1). The increase was significant from the sixth day of exposure until the end of the experiment. The intact crabs in clean water showed no significant changes. In contrast, the glucose concentrations in the blood of the eyestalk-ablated crabs in clean water (Group 1C) and cadmium-containing water (Group 1D) did not change significantly throughout the experimental period nor did Groups 1C and 1D differ significantly from each other at any time.

The blood glucose concentration in the naphthalene-exposed intact crabs (Group 2B), like the cadmium-exposed crabs, increased significantly after 6, 8 and 10 days of exposure compared with crabs in clean water (Group 2A) (Table 2). In contrast, the eyestalkless crabs in naphthalene-containing water (Group 2D) as well as in water without naphthalene (Group 2C) showed no significant changes in their blood sugar level, nor did Groups 2C and 2D differ significantly from each other at any time. The blood glucose concentrations in Experiment

Table 2. Blood glucose levels (mg glucose per 100 ml blood) of intact and eyestalk-ablated fiddler crabs, *Uca pugilator.* See text for key to groups. Values are given as mean ± SEM. N=20. *: P<0.001, versus control.

	Exposure period (Days)				
	2	4	6	8	10
Group 2A	7.16 ± 0.60	7.90 ± 0.52	8.74 ± 0.52	8.16 ± 0.32	8.50 ± 0.68
Group 2B	8.74 ± 0.56	8.70 ± 0.76	13.24 ± 1.00*	13.60 ± 0.84*	14.80 ± 3.10*
Group 2C	8.06 ± 0.52	8.32 ± 0.60	9.16 ± 0.38	7.80 ± 0.50	8.88 ± 0.54
Group 2D	8.20 ± 0.62	8.40 ± 0.66	7.92 ± 0.58	7.56 ± 0.58	8.16 ± 0.56

II are lower than the corresponding ones in Experiment I. Other than the fact that different shipments of crabs were used for each experiment, we have no explanation for the difference.

Experiment III was performed to determine the effects of 10 days of exposure to cadmium or naphthalene on CHH activity in the eyestalks. Eyestalk extracts of crabs from clean water (Group 3A), cadmium-exposed (Group 3B) and naphthalene-exposed (Group 3C) crabs were injected into intact crabs (Groups E, F and G, respectively) and a fourth intact group (Group D) received physiological saline. After 2 hr the blood glucose concentrations were determined (Table 3). Eyestalks of cadmium-exposed crabs produced significantly less hyperglycemia (Group 3F) than did eyestalks of crabs from clean water(Group 3E). In contrast, eyestalks of naphthalene-exposedcrabs produced significantly more hyperglycemia (Group 3G) than eyestalks of crabs from clean water (Group 3E).

Pollutant-induced hyperglycemia was also reported by Fingerman et al. (1981) with DDT for the crab, *Barytelphusa guerini,* Nagabhushanam and Kulkarni (1981) with cadmium, mercury and copper for the prawn, *Macrobrachium kistnensis,* and Reddy et al. (1994) with cadmium for *Procambarus clarkii.* In contrast to the intact fiddler crabs (Tables 1 and 2), the eyestalkless ones exposed to cadmium and naphthalene did not show an increase in blood glucose. This difference between the responses of intact and eyestalkless crabs strongly suggests the involvement of CHH in the cadmium- and naphthalene-induced hyperglycemia in the intact crabs.

Table 3. Blood glucose levels (mg glucose per 100 ml blood) of intact fiddler crabs, *Uca pugilator,* two hours after injection with physiological saline or eyestalk extract. See text for key to groups. Values are given as mean ± SEM. N=20.

Treatment group	Blood glucose level
Group 3D	11.40 ± 0.60
Group 3E	48.50 ± 3.50
Group 3F	22.80 ± 1.60
Group 3G	82.40 ± 4.50

The present findings suggest that irrespective of the type of pollutant (heavy metals or hydrocarbons), upon exposure crustaceans exhibit hyperglycemia that is mediated by CHH from the eyestalks.

In crayfishes, eyestalk ablation results in hypoglycemia (Keller and Beyer 1968; Reddy et al. 1994), but with eyestalkless crabs, as herein, no significant hypoglycemia occurs (Abramowitz et al. 1944; Lüschen et al. 1993).

The hyperglycemic activity of the eyestalk extracts of the cadmium-exposed crabs was less than that of the crabs kept in clean water, whereas the extracts from naphthalene-exposed crabs were more active (Table 3). These results suggest that even though both naphthalene and cadmium induce hyperglycemia, their modes of action are different. The hyperglycemia in the intact crabs exposed to cadmium and naphthalene was presumably due to released CHH. The different amounts of CHH activity in the eyestalks of the crabs exposed to cadmium and naphthalene relative to the eyestalks of the crabs in clean water (Table 3) suggest that in the long term cadmium inhibits CHH synthesis whereas naphthalene stimulates CHH synthesis. Reddy and Fingerman (1995), using histochemical techniques, observed that cadmium exposure resulted in depletion of the neurosecretory material in the eyestalk neuroendocrine cells of *Uca pugilator,* which is consistent with the suggested inhibitory mode of action of cadmium on BPDH and CHH synthesis. Deecaraman and Fingerman (1985) observed an increase in the neurosecretory material in the brain of *Uca pugilator* exposed to naphthalene, and Nagabhushanam et al. (1979) reported Aroclor 1242 exposure produced an increase in the amount of neurosecretory material in the medulla terminalis X-organ of *Uca pugilator.* Consistent with these histological studies are the observations with

Uca pugilator that Aroclor 1242 (Fingerman and Fingerman 1978) and naphthalene (Staub and Fingerman 1984) produced increases in stored BPDH. These four studies are also consistent with the observation (Table 3) that the eyestalks of the naphthalene-exposed crabs showed more CHH activity than did the eyestalks of the crabs in clean water. It seems evident, therefore, that exposure to organic pollutants leads to accumulation of neurohormones in the eyestalk neuroendocrine complex. In contrast, exposure to cadmium, an inorganic, produces depletion of these neurohormones.

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