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Effects of Cadmium and Copper on Hormonal Regulation of Glycemia by the Eyestalks in the Crab *Chasmagnathus granulata*

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The X-organ-sinus gland complex in the eyestalks of decapod crustaceans is their principal endocrine center (Fingerman, 1987). One of the hormones secreted by this neuroendocrine complex is the crustacean hyperglycemic hormone or CHH. This hormone has been isolated from several species and then purified and sequenced (Keller, 1992). The relevant stimuli for its secretion and the mechanisms of action of this hormone have already been reported (Santos and Keller, 1993). Although the presence of CHH in the estuarine crab *Chasmagnathus granulata* is well-known (Nery *et al.*, 1993), this hormone has not been purified yet in this crab.

Changes of temperature or salinity, anoxia, deprivation of food and exposure to air are some of the factors that can act as stressors in crustaceans. Environmental pollutants such as pesticides and heavy metals are also typical stressors in crustaceans that can result in hormonal imbalances (Fingerman *et al.*, 1996). Crustaceans typically display hyperglycemia in response to adverse conditions. In this sense, CHH seems to play a role in crustaceans similar to that of glucocorticoids in vertebrates. The variety of mechanisms and targets for heavy metal toxicity could produce, though, a different response, depending on heavy metal concentrations and time of exposure, among other factors.

Reddy *et al.* (1994, 1996) reported that cadmium could cause a decrease of CHH synthesis, probably related to the inhibition that this metal might exert on enzymes involved in the synthesis of this hormone. The interaction of heavy metals with enzymes and other proteins has been widely studied, even in invertebrates (Rainbow and Dallinger, 1993). The present study was aimed at studying the effect of chronic exposure to cadmium and copper on CHH-mediated glycemia in the crab *C. granulata*.

MATERIALS AND METHODS

Adult males *Chasmagnathus granulata* were collected at Faro San Antonio Beach, the southern limit of Samborombón Bay, Argentina (36°18'S, 56°48'W) in October 1999. Once in the laboratory, they were acclimated for

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15 d to the same environmental conditions to be used during the bioassays, namely a temperature of 20 ± 1°C, a photoperiod of 14:10 (L:D) and 12 ‰ salinity.

The crabs used in the experiments had an average carapace width of 28.9581 ± 0.1103 mm (n=155) of carapace width. Fifteen crabs were randomly assigned to glass aquaria filled with 5 L of clean saline water under continuous aeration, with a known concentration of toxicant added. In all cases, saline water was prepared with salts for artificial sea water (Marinemix HW, Germany) and dechlorinated tap water (hardness: 80 mg/L of $CaCO_3$ equivalents).

For the first experiment, the following experimental groups were set up for each of the assayed heavy metals,:
1A: intact crabs in clean water
1B: intact crabs exposed to 0.5 mg/L cadmium or 0.1 mg/L copper
1C: eyestalk-ablated crabs in clean water
1D: eyestalk-ablated crabs exposed to 0.5 mg/L cadmium or 0.1 mg/L copper

The cadmium and copper concentrations used represent 1/50 of the respective 96 h-LC50 values for this crab (Rodríguez *et al.*, 1998). Eyestalks were ablated at their base by means of fine scissors, and the wound was then cauterized to prevent excessive bleeding. This experiment lasted two weeks; during this period crabs were fed twice a week, *ad libitum*, rabbit pellet food, as was previously done in experiments with the same species (Rodríguez *et al.*, 1992). All test solutions were renewed after each feeding. At the end of the experiment, 200 µL of hemolymph was taken from the base of the fourth or fifth pereiopod, by use of a 1 mL syringe (27G needle). Glycemia was determined by means of the glucose oxidase method (kit from Wiener Lab.)

In order to confirm the effect of a wider range of concentrations of each metal, additional concentrations were run with intact crabs. Therefore, the following concentration series were defined, for cadmium: 0, 0.05, 0.1, 0.25 and 0.5 mg/l and for copper: 0, 0.05, 0.1 and 0.25 mg/L. Exposure time and all other experimental conditions, including determination of glycemia, were the same as stated above.

For both, cadmium and copper, the following groups were run in the second experiment:
2A: Intact crabs, injected with crustacean saline (Cooke *et al.*, 1977)
2B: Intact crabs, injected with eyestalk extracts made from crabs of group 1A
2C: Intact crabs, injected with eyestalk extracts made from crabs of group 1B

This second experiment began immediately after the first concluded. The extracts were prepared by homogenizing the eyestalks with crustacean saline (Cooke *et al.*, 1977), they were later centrifuged for 10 minutes at 10,000 g, 4 °C. Fifty µL of the supernatant (representing 0.5 eyestalk equivalent) was injected into each crab in groups 2B and 2C, while 50 µL of crustacean saline was injected into crabs of group 2A. Injections were made in the dorsal articulation of the cephalothorax and pleon. After 60 minutes, time enough for a hyperglycemic response to occur in *C.granulata* after CHH administration (Nery *et al.*, 1993), a sample of hemolymph was taken from each crab, and analyzed in the same way as in the first experiment.

The results obtained from both experiments were analyzed by means of a one-way ANOVA, followed by planned comparisons (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Table 1. Mean hemolymphatic glucose levels (mg/100mL) ± standard error. First experiment. Same letters indicate no significant differences ($p>0.05$) within the assay for each heavy metal.

Experimental Group	Glucose level	
	Cadmium	Copper
1A	8.85 ± 1.50	8.45 ± 0.70
1B	4.35 ± 0.34 (a)	3.34 ± 0.45 (b)
1C	4.78 ± 0.45 (a)	3.20 ± 0.44 (b)
1D	4.97 ± 0.50 (a)	3.46 ± 0.45 (b)

Table 1 shows the results obtained from the first experiment. For both heavy metals assayed, the exposed intact crabs (1B group) showed a significant ($p<0.05$) decrease of glycemia, as compared to the to intact crabs in clean water (1A group), reaching in fact a glucose level similar to that of the eyestalkless crabs (groups 1C and 1D). These results strongly suggest that the effects of both cadmium and copper resulted in a drastic inhibition of CHH secretion by the sinus gland in the eyestalks. The hyperglycemic response expected from the fact that both heavy metals assayed are strong stressors, as mentioned in the Introduction, seems to have been abolished by the same metals specifically inhibiting the secretion of the hyperglycemic hormone, at least in the concentrations and time of exposure used in this study. However, the possibility of some interaction of cadmium or copper with CHH receptors in several tissues (as suggested for Reddy *et al.*,1994) should not be discarded for explaining the hypoglycemic responses.

A marked hyperglycemia caused by cadmium and mediated by CHH, has in turn been reported in the crayfish *Procambarus clarkii* (Reddy *et al.*, 1994), as well as in the fiddler crab *Uca pugilator* (Reddy *et al.*, 1996). *P. clarkii* was exposed up to 72 h to 5 mg/L cadmium, with the hyperglycemic response being maximal at 48 h. In the case of *U. pugilator*, a longer time of exposure was maintained (up to 10 d, similar to the time used in this work); the difference in the response, compared to that of *C. granulata*, could be related to the concentration used, since *U. pugilator* was exposed to 5 mg/L cadmium while *C. granulata* was exposed to a cadmium concentration ten-fold lower. Further experiments are needed to elucidate this point, taking into account the particular features of both species.

Since no differences ($p>0.05$) were detected in any case between control and heavy metals-exposed eyestalkless crabs, no direct effect of the assayed pollutants on basal glycemia levels (not regulated by CHH) seems to have occurred. However, the possibility of a more effective clearing of heavy metals by the excretory organs of eyestalkless animals, compared to intact ones, should not be discarded.

The clear hypoglycemia (Table 1), produced by both cadmium and copper was in fact verified in intact crabs exposed to a wider concentration range (Figure 1). A typical dose-response relationship was observed for both heavy metals. Crabs showed a higher sensitivity to copper than to cadmium, according to the concentrations that produced a significant drop of glycemia, with respect to the control. This response was consistent with the lower acute LC50 value for copper than for cadmium, as mentioned above, although the difference in the chronic toxicity on glycemia between both heavy metals (a factor of ± 2, from Fig. 1) was not as high as the difference in lethal acute toxicity (a factor of 5). This is probably due to the unregulated mechanisms of cadmium accumulation, that could lead to continuous increasing accumulation as exposure time increases (Rainbow and Dallinger, 1993).

Table 2. Mean hemolymphatic glucose levels (mg / 100ml) ± standard error. Second experiment. Same letters indicate no significant differences ($p>0.05$) within the assay for each heavy metal.

Experimental Group	Glucose level	
	Cadmium	Copper
2A	6.32 ± 1.11	5.51 ± 1.02
2B	45.38 ± 3.68 (a)	47.18 ± 5.29 (b)
2C	42.78 ± 3.51 (a)	47.07 ± 5.26 (b)

Table 2 shows the results from the second experiment. Slight differences in glucose level noted between control groups 1A and 2A were probably due to normal daily variation reported for crustaceans (Arechiga *et al.*,

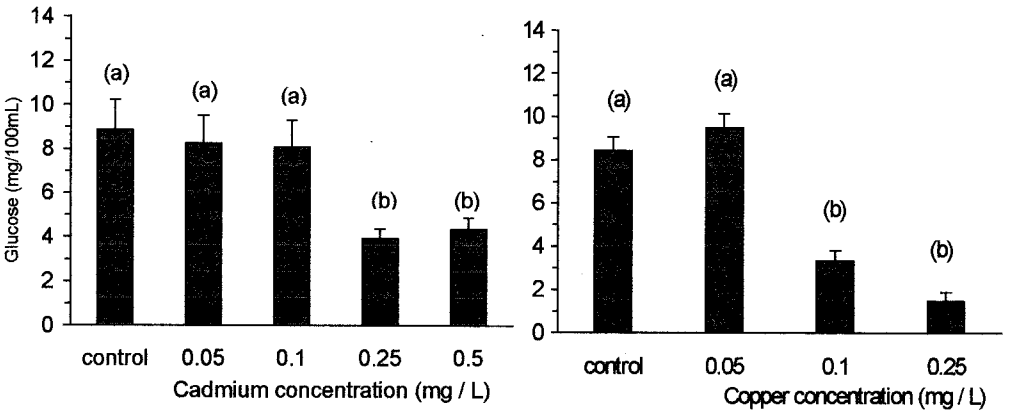

Figure 1. Mean hemolymphatic glucose levels (mg/100mL) ± standard error, for intact crabs exposed to several concentrations of cadmium or copper (dose-response curve). Same letters indicate no significant differences ($p>0.05$)

1993). The very significant differences ($p<0.01$) detected between the group injected with saline solution (2A) and the group injected with eyestalk extract from crabs in clean water (2B) clearly show the high hyperglycemic effects of the CHH normally present in the sinus glands of the control animals. The group injected with eyestalk extract from crabs previously exposed to cadmium or copper (2C) showed the same level of glycemia as that of the respective groups, indicating that no apparent effect of either cadmium or copper in the content of CHH in the eyestalks seems to have occurred.

Although, the hypoglycemia produced by both metals was probably due to inhibition of CHH secretion, any consequent accumulation of this hormone in the sinus gland was not apparent, perhaps there was a compensatory decreased rate of CHH synthesis in the exposed crabs. Inhibition of CHH synthesis by cadmium has already been reported for the crab *Uca pugilator* (Reddy *et al.,* 1996). Nevertheless, the regulatory mechanisms of CHH turnover in the neurons that synthesize CCH could also be maintaining a steady state of CHH availability, i.e., reducing synthesis when secretion of the hormone decreases.

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