Bull. Environ. Contam. Toxicol. (2004) 73:942–946

DOI: 10.1007/s00128-004-0517-7

Bulletin of Environmental Contamination and Toxicology

Disruption of Endocrine Regulation of Glycemia Levels by Cadmium and Copper in the Estuarine Crab *Chasmagnathus granulata*

D. A. Medesani, L. S. López Greco, E. M. Rodríguez

Department of Biodiversity and Experimental Biology, FCEN - University of Buenos Aires, Ciudad Universitaria, Pab. II, C1428EHA Buenos Aires, Argentina

Received: 13 November 2003/Accepted: 30 July 2004

Carbohydrate metabolism has been one of the topics extensively studied in crustaceans (see Santos and Keller 1993a, for review). Hormonal control of circulating levels of glucose has also been one of the best-studied subjects in the field of crustacean endocrinology. The so-called "crustacean hyperglycemic hormone" (CHH) is a peptide of about 70 amino acids, that has been isolated and sequenced for several crustacean species (Keller 1992). It belongs to a family of neuropeptides, all of them released from the sinus gland in the eyestalks (Charmantier et al. 1997). Hyperglycemia in situations such as the emersion of crabs has been reported (Santos and Keller 1993b; Schmitt and Santos 1993) in correlation with increased levels of circulating CHH (Webster 1996). Since other stimuli promote CHH secretion, this hormone has been also proposed to act as a stress hormone for crustaceans (Chang et al. 1998, 1999). On the other hand, reproductive functions of some isoforms of CHH have been also reported and reviewed (Charmantier et al. 1997; De Kleijn and Van Herp 1998).

A hyperglycemic response has also been reported in crustaceans due to exposure to heavy metals such as cadmium (Reddy et al. 1994, 1996) and some other pollutants (Fingerman et al. 1998). However, a hypoglycemic dose-dependent effect was reported for the South American estuarine crab *Chasmagnathus granulata* after a 2-week exposure to cadmium and copper (Medesani et al. 2001). Hypoglycemia was seen in intact crabs, but not in eyestalkless ones. From these results, we proposed the hypothesis that the observed hypoglycemia was caused by both heavy metals through inhibition of CHH secretion from the eyestalks. However, an alternative hypothesis that those heavy metals antagonize the hormone action at target tissues merits investigation. This study is aimed therefore at testing this latter hypothesis.

MATERIALS AND METHODS

Adult males were collected at the Faro San Antonio beach (36°18'S, 56°48'W) on July 6, 2001. Once in the laboratory, the crabs were acclimated to laboratory conditions for 2-week, i.e., a temperature of 20°C ± 1°C, photoperiod 14:10 (L:D) and a salinity of 12 ‰ (Marinemix aquarium salts in dechlorinated tap water at a hardness of 80 mg/L of $CaCO_3$ equivalents, final pH 7.8). These environmental conditions were the same as the ones maintained throughout this study.

Correspondence to: E. M. Rodríguez

Sixty crabs (mean weight: 17.49 ± 0.41 g) were randomly separated into three groups of 20 crabs. Each group was placed in a glass container filled with 5 L of 12 ‰ saline water, under constant aeration, and was randomly assigned to each of the following experimental treatments:

CD: exposed to 0.5 mg/L of cadmium
CU: exposed to 0.1 mg/L of copper
CTRL: with only the saline water used in all groups

Cadmium chloride and copper sulphate of analytical grade purity were used. The concentrations used for both heavy metals were 1/50 of the corresponding 96 h-LC50 values reported for this species (Bigi et al. 1996; López Greco et al. 2001). Furthermore, those concentrations were close to the ones that did not cause any effect on glucemia in the studied species for the same time of exposure, according to the dose-response study previously carried out (Medesani et al. 2001). During the assays, the crabs were fed rabbit pellet food twice a week, as in previous studies with the same species (Rodríguez et al. 1992; López Greco et al. 2001; among others). All test solutions were replaced after feeding.

After the two weeks of exposure, the crabs from all the experimental groups were injected with either crustacean saline (Cooke et al. 1977) or CHH (32 pmoles/crab) purified from *Cancer pagurus* (Webster 1996) and dissolved in crustacean saline. The injection volume was always 50 µL, inyected into the base of the 4th pair of periopods by means of a 27G needle. Sixty minutes after the crab were injected, a sample of hemolymph (200 µL) was withdrawn from the injection site, and the glycemia level was measured by means of the glucose oxidase method (Wiener Lab. Kit), the absorbance being measured at 505 nm. The time for taking the sample after injection was selected according to previous studies made on the same species injected with exogenous CHH (Nery et al. 1993). Doses of CHH of 16 and 64 pmoles/crab were assayed in a preliminary experiment designed to determine the appropiate amount of CHH to use in these experiments. The obtained results were compared by means of a one-way ANOVA, followed by planned comparisons (Sokal and Rohlf 1981). Data for deaths that occurred during the assay were analyzed by the Fisher exact test (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Concerning mortality, no significant differences ($p>0.05$) were observed among the experimental groups; the highest mortality (20 %) ocurred in cadmium-exposed group. Figure 1 shows the mean glycemia values recorded for the three groups after the 2-week exposure. Both cadmium and copper produced significantly ($p<0.05$) lower glycemia levels as compared with the control group.

On the other hand, injection of CHH into both the control and heavy metals exposed crabs showed a significant ($p<0.05$) increase of the glycemia, as compared to their respective saline groups (Fig. 1). Of the three groups of crabs injected with CHH, the cadmium group did not differ ($p>0.05$) from the control group, whereas the copper

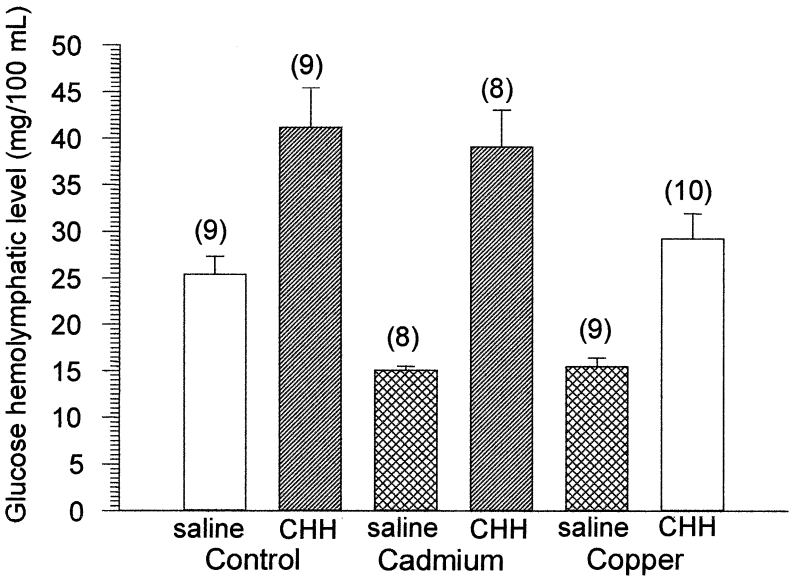

Figure 1. Mean glycemia levels in *C. granulata* at the end of the experiment. Standard errors are also indicated. Number of crabs is indicated between brackets. Different bar fills indicate significant differences ($p<0.05$).

group did ($p<0.05$), its mean glycemia increasing to a value similar ($p>0.05$) to that of the control crabs injected with saline.

Both heavy metals induced in the crabs that subsequently were given an injection of the saline solution significant hypoglycemic responses (Fig. 1). This result was similar to that previously reported for the same species with the same heavy metal concentrations, time of exposure and other experimental conditions (Medesani et al. 2001); with even a similar reduction (50 to 60 %) of the glucose levels having been observed in both cases.

However, studies made on the crayfish *Procambarus clarkii* (Reddy et al. 1994) and the crab *Uca pugilator* (Reddy et al. 1996) showed a hyperglycemia after exposure to cadmium. In the case of *P. clarkii*, the concentration used (5 mg/L) was 10-fold higher than that used in the current study, but even more relevant is the fact that an acute exposure (72 hours) was carried out in the former study, in contrast to the 2-week exposure period maintained for *C. granulata*. For *U. pugilator*, the same high concentration of cadmium was assayed, but for a period up to 10 days. Interestingly, a further study made on the same species showed that cadmium could increase the secretion of GIH, an eyestalk peptide member of the same family as CHH, just as it appeared to trigger CHH release, while recent results obtained in *C. granulata* indicate an inhibitory effect of both cadmium and copper on GIH secretion (Medesani and Rodríguez, unpublished) just as these metals appear to inhibit CHH secretion. These results appear to reflect species differences among crustaceans.

CHH of *Carcinus maenas* has been injected into *C. granulata* from Brazil (Nery et al. 1993) at a dose of 16 pmoles/crab (crabs averaging 8 g of wet weight), with an increase in glycemia similar that to observed in the current study at a dose of 32 pmoles/crab with crabs averaging 17 g. A circulating level of endogenous CHH was reported for the crab *Cancer pagurus* as ranging from 17 to 30 pmol/L (Webster 1996). Administration of CHH from *Cancer pagurus* was able to completely reverse the hypoglycemia caused by cadmium in *C. granulata*, increasing the glycemia in the cadmium-exposed crabs to the same level as that observed in the control crabs after administration of the same doses of CHH. This result is evidence that cadmium is not affecting the CHH receptors at target tissues and/or the transduction pathways of the hormonal effect, and strongly supports the hypothesis that cadmium is causing the hypoglycemia by inhibiting CHH secretion.

In the case of the crabs exposed to copper, the injection of CHH significantly increased the glycemia over the reduced levels observed after the 2-week exposure period. However, the glycemia that was observed was less than that which ocurred in the control crabs injected with CHH at the same dose, suggesting that, at least in part, copper could be affecting the CHH receptors and/or transduction in the target tissues, i.e., muscle, hepatopancreas, epidermis, among others (Santos and Keller 1993). Because CHH would exert its hyperglycemic effect by stimulating glycogen degradation in several tissues, as well as other carbohydrate pathways, a possible inhibitory effect of copper on enzymes involved in those pathways should also be taken into account.

Acknowledgments. We thank Dr. Simon Webster for his gift of the *Cancer pagurus* CHH, and to Dr. Milton Fingerman for his critical reading of the manuscript. This work was supported by grants from CONICET (PIP0917) and ANPCYT (PICT03227).

REFERENCES

Bigi R, Verrengia-Guerrero N, Rodríguez EM, Kesten E, Medesani DA (1996) Acute lethal toxicity and bioaccumulation of cadmium in the estuarine crab *Chasmagnathus granulata* (Decapoda, Brachyura). In: Marcovecchio J (ed) Pollution Processes in Coastal Environments, Mar del Plata, p 292

Cooke IM, Haylett BA, Weatherby TM (1977) Electrically elicited neurosecretory and electrical responses of the isolated crab sinus gland in normal and reduced calcium salines. J Exp Biol 101:125-149

Chang ES, Keller R, Chang SA (1998) Quantification of crustacean hyperglycemic hormone by ELISA in hemolymph of the lobster *Homarus americanus* following various stresses. Gen Comp Endocrinol 111:359-366

Chang ES, Chang SA, Keller R, Reddy PS, Snyder MJ, Spees JL (1999) Quantification of stress in lobsters: Crustacean hyperglycemic hormone, stress proteins, and gene expression. American Zool 39:487–495

Charmantier G, Charmantier-Daures M, Van Herp F (1997) Hormonal regulation of growth and reproduction in crustaceans. In: Fingerman M, Nagabhushanam R and Thompson MF (eds) Recent Advances in Marine Biotechnology, Vol 1. Oxford & IBH Publishing, New Delhi, p 109

De Kleijn DPV, Van Herp F (1998) Involvement of the hyperglycemic neurohormone family in the control of reproduction in decapod crustaceans. Invert Reprod Dev 33:263–272

Fingerman M, Jackson N, Nagabhushanam R (1998) Hormonally-regulated functions in crustaceans as biomarkers of environmental pollution. Comp Biochem Physiol 120C:343-350

Keller R (1992) Crustacean neuropeptides: structures, functions and comparative aspects. Experientia 48:439–448

López Greco LS, Sánchez MV, Nicoloso GL, Medesani DA, Rodríguez EM (2001) Toxicity of cadmium and copper on larval and juvenile stages of the estuarine crab *Chasmagnathus granulata* (Decapoda, Grapsidae). Arch Environ Contam Toxicol 41:333-338

Medesani DA, López Greco LS, Rodríguez EM (2001) Effects of cadmium and copper on hormonal regulation of glycemia by the eyestalks, in the crab *Chasmagnathus granulata*. Bull Environ Contam Toxicol 66:71-76

Nery LEM, Santos EA, Bianchini A, Gonçalves AA (1993) Effects of crustacean hyperglycemic hormones from *Carcinus maenas* and *Orconectes limosus* on blood and muscle glucose and glycogen concentration of *Chasmagnathus granulata.* Brazilian Med Biol Res 26:1291–1296

Reddy PS, Devi M, Sarojini R, Nagabhushanam R, Fingerman M (1994) Cadmium chloride induced hyperglycemia in the red swamp crayfish, *Procambarus clarkii*: possible role of crustacean hyperglycemic hormone. Comp Biochem Physiol 107C:57–61

Reddy PS, Katyayani RV, Fingerman M (1996) Cadmium and naphthalene induced hyperglycemia in the fiddler crab, *Uca pugilator*: differential modes of action on the neuroendocrine system. Bull Environ Contam Toxicol 56:425–431

Rodríguez EM, Monserrat JM, Amín OA (1992) Chronic toxicity of ethyl parathion and isobutoxyethanol ester of 2,4-dichlorophenoxyacetic acid to estuarine juvenile and adult crabs. Arch Environ Contam Toxicol 22:140-145

Santos EA, Keller R (1993a) Crustacean hyperglycemic hormone (CHH) and the regulation of carbohydrate metabolism: current perspectives. Comp Biochem Physiol 106A:405–411

Santos EA, Keller R (1993b) Effect of exposure to atmospheric air on blood glucose and lactate concentration in two crustacean species: a role of the crustacean hyperglycemic hormone (CHH). Comp Biochem Physiol 106A:343-347

Schmitt ASC, Santos EA (1993) Lipid and carbohydrate metabolism of the intertidal crab *Chasmagnathus granulata* Dana, 1851 (Crustacea, Decapoda) during emersion. Comp Biochem Physiol 106A:329-336

Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research, 2nd ed. WH Freeman and Company, New York

Webster SG (1996) Measurement of crustacean hyperglycaemic hormone (CHH) level in the edible crab, *Cancer pagurus* during emersion stress. J Exp Biol 199:1579-1585