THE MOLECULAR MECHANISMS OF THE REGULATION OF THE HEAMOLYMPH GLUCOSE LEVEL IN THE CRAB *(CARCINUS ESTUARIS)*

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Abstract

Hyperglycemia is a typical response of many aquatic animals to pollutants and stress and in crustaceans; increased circulating cHH and hyperglycemia are reported to result from exposure to several environmental stressors. Biogenic amines and encephalin have been found to mediate the release of several neurohormones from crustacean neuroendocrine tissue and a model of the controlling network is proposed.

The purpose of this study has been the investigation of mechanisms of glucose regulation in crustacean crab *Carcinus estuaries*, for the first time in Albania. The haemolymph glucose levels in individuals of a control and treatment groups of crabs has been measured. Animals have been purchased from a local commercial fisherman in the Narta lagoon during the period March- May, 2011-2012. Also, the glucose level after the eyestalk extract injection and after the adrenaline injection has been determinate. It has been shown that the eyestalk ablation of the crabs lead to a significantly decreasing in the haemolymph glucose level (38.68±3.01 mg/dl to 14.63±1.19 mg/dl, $F=55.04$, $df=1.10$, $p<0.05$) compared with the intact animals (control group; 38.68±3.01 mg/dl to 42.95±3.42 mg/dl, $F=0.877$, $df=1.10$, $p>0.05$). The level of haemolymph glucose in the eyestalk-ablated animals has been increased drastically after the eyestalk extract injection (14.63±1.19 mg/dl to 61.52±3.21 mg/dl, $F=187.19$, $df=1.10$, $p<0.05$) while in the normal animals it doesn't produce any significant effect (38.68±3.01mg/dl to 42.95±3.42 mg/dl, $F=0.877$, $df=1.10$, $p>0.05$). The injection of adrenaline has been also induced a drastically increasing of the haemolymph glucose level (39.3±2.15 mg/dl to 50.15±2.57 mg/dl, $F=8.298$, $df=1.10$) and a slightly decreasing of the haemolymph glucose level in the eyestalk-animals (15.77±3.49 mg/dl to 14.63±2.16 mg/dl $F=0.077$, $df=1.10$, $p>0.05$). In conclusion, blood glucose level in crustaceans is controlled by the crustacean Hyperglycemic Hormone (cHH), released from the eyestalk neuroendocrine centers both under physiological and environmental stress conditions.

Key words: *Carcinus estuaris, adrenaline, eyestalk, crustacean hyperglycemic hormone(CHH), glucose.*

Introduction

Hyperglycemia is a typical response of many aquatic animals to harmful physical and chemical environmental changes. In crustaceans increased circulating crustacean Hyperglycemic Hormone (cHH) titres and hyperglycemia are reported to occur following exposure to several environmental stressors (Durand *et al.*, 2000; Lorenzon *et al.*, 1997; 2002; Santos *et al.*, 2001) in intact but not in eyestalkless animals , suggesting a cHH mediated respons (Fingerman *et al.*, 1981; Reddy and Bhagyalakshimi,1994; Reddy *et al.*, 1996; Lorenzon *et al.*, 2000, 2004a).

Toxicity induced by a pollutant is the result of interaction of the compound or one of its metabolites, with the biochemical events involved in the homeostatic control of aphysiological process (Brouwer *et al.*, 1990). Physiological processes are mostlycoordinated by hormones.

In crustaceans, the X-organ/sinus gland complex typically located in the eyestalk plays a central role in the physiological regulation of biological activities (Fingerman, 1987; Keller, 1992). Hormones produced by this system are known to regulate reproduction, metabolism, osmoregulation, chromatic adaption and growth. One of the best-known crustacean hormones with a relatively fast response time (between 1 and 2 h for maximum response) is the crustacean hyperglycemic hormone (Santos and Colares, 1986, 1990; Keller and Sedlmeier, 1988). The crustacean hyperglycemic hormone is involved in the regulation of hemolymph glucose, lipids and hepatopancreatic enzyme secretion. The crab, Carcinus estuaries is potent source of food and it has rich amount of nutrients. It is commercially important crab along Adriatic coast and abundant throughout the year. Very limited study was carried out on the role of eyestalk hyperglycemic hormone in the hemolymph glucose levels of crabs in general and none study was make in Carcinus esuaris in Albania. The present study is aimed to estimate the total free and reducing sugars of crab' s hemolymph before and after eyestalk ablation and after adrenaline injection.

The crustacean Hyperglycemic Hormone (cHH)

Multiple forms of the cHH represent one member of an eyestalk neuropeptide family (Bocking *et al*, 2001), that includes the moult inhibiting hormone(MIH) and the gonad inhibiting hormone (GIH): the cHH/MIH/GIH family. These neuropeptides, synthesized in the XO, a cluster of neuron perikarya located in the medulla terminalis of the eyestalk, are transported to and stored in the axon terminals forming a neurohemal organ named SG and released by exocytosis into the hemolymph. The main function of cHH is the regulation of hemolymph sugar level: cHHs are also involved in other functions such as reproduction (De Kleijn *et al.*, 1998; De Kleijn and van Herp, 1998), molting (Chung *et al.*, 1999; Webster *et al.*, 2000), lipid metabolism (Santos *et al.*, 1997), stress response (Lorenzon *etal.*, 1997; 2002;Chang *et al.*, 1999; Durand *et al.*, 2000; Santos *et al.*, 2001) and hydromineral regulation (Spanings-Pierrot *et al.*, 2000; Serrano *et al.*, 2003). On the basis of the primary structure, thecHH/MIH/GIH family can be divided into two subfamilies(De Kleijn *et al.*, 1995; Lacombe *et al.*, 1999):the cHH sub-family characterized by the

cHHprecursor-related peptide (CPRP) and the MIH/GIH sub-family without CPRP. The prepropeptide cHHconsists of a signal peptide, CPRP.

Materials and methods

Healthy crabs were brought to the laboratory from the lagoon of Nart and acclimatized in the laboratory conditions (see water 35‰, temperature 17 °C during the day and 10°C during the night).Crayfishwere maintained in the laboratory in tanks through which tap water circulated, adepth of about two inches of water being maintainedand were fed with freshly-killed fish for 3 days. Total free and reducing sugar were determinated in intact control crabs(6 individs). Subsequently eyestalk ablation was performed in the same animals by cutting the eyestalks at its base with clean scissors and the wound was cauterized with a hot blunt needle in order to prevent the loss of hemolymph and mortality (Caillouet, 1973). The hemolymph was collected by hypodermic syringe. Glucose was estimated in both groups of animals by oxydase peroxydase method.

Eystalk exstract was prepared by grinding 4 eystalks with 0,5 ml of distilled water in a mortar and a small amount of clean see sand. This extract was centrifuged for 5 minutes to settle the coarse detritus. The supernatant was separated and used for the eksperiment. The experimental groups were divided into groups with 6 individs: one group with eyestalks and one group without eyestalks. The initial hemolymph was collected from the intact control and eyestalk ablated crabs and determines the glucose level by the glucose oxydase method. After that 0.1 ml of the crude eyestalk was injected to both set of experimental animals and allowed to stand for sometime. After 2 h , the hemolymph was collected again to both intact control and eyestalk ablated animals and the glucose was estimated as earlier.

The animals are devided into 2 groups. One group served as intact control group and other group was eyestalk-ablated animals. Before theinjection of adrenaline, the hemolymph samples were taken in both groups and glucose was estimated. The animals were exposet to adrenaline stress for 1.5 h and then was collected the hemolymph to estimate the glucose level.

RESULTS

Rezults of ekstrakt injection:

The crude eyestalk injection increasedrasticly the glucose level in the hemolymph of crabs. The maximum level of glucose is arrised 2 h after the injection (Abramowitz *et al.*, 1944; Keller and Andrew, 1973; Keller*et al.*, 1985; Santos and Keller, 1993b).The result of the experiment 1 are presented in TABLE 1.In the control group was noticed that the glucose level was 30-48 mg/dl haemolymph.

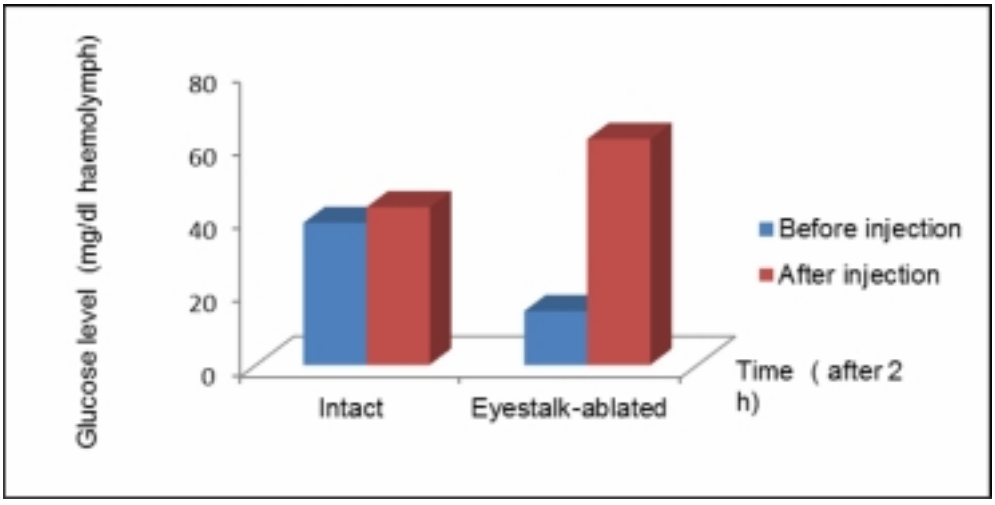
TABLE 1

Animals	Nr	Before injection	After injection
Intact	6	38.68±3.01	42.95±3.42^{NS}
Eyestalk-ablated	6	14.63±1.19***	61.52±3.21***

The glucose level was drasticly decreased after the eyestalk"s ablation from 38.68±3.01 mg/dl haemolymph to 14.63±1.19 mg/dl haemolymph (F=55.04, df=1, 10, p=0.000 so p<0.05).

In both the groups, an increase in the glucose level was noticed 2 h after the eyestalk was injected. The eyestalk extract injection increases drastically the level of glucose in haemolymph eyestalk-ablated animals from 14.63±1.19 mg/dl haemolymphto 61.52±3.21*** mg/dl haemolymph (F=187.19, df=1,10, p=0.000 so p<0.05) while in the intact animals since CHH was already mantaing the glucose level of hemolymph, the exess addition of eyestalk extract doesn't produse any significant effect from 38.68±3.01mg/dl haemolymphto 42.95±3.42^{NS} mg/dl hemolymph (F=0.877, df=1,10, p=0.371 so p>0.05).

Fig 1: Rezults of ekstrakt injection



Rezults of adrenaline injection

The injection of adrenaline increase drasticly the glucose level in the hemolymph of intact crabs. The maximum level of glucose is arrised 1,5 after the injection. The result of the experiment are presented in TABLE 2.

The glucose level was drasticly increased after the adrenaline injection in the intact crabs from39.3±2.15 mg/dl haemolymphto 50.15±2.57* mg/dl haemolymph (F=8.298, df=1, 10, p=0.016 so p<0.05) while in haemolymph eyestalk-ablated animals the adrenaline injection doesn't produce any significant effect from 15.77±3.49 mg/dl haemolymph to 14.63 ± 2.16^{NS} mg/dl haemolymph (F=0.077, df=1,10, p=0.787 so p>0.05)

TABLE 2

Animals	Nr.	Before injection	After injection
Intact	6	39.3±2.15	50.15±2.57*
Eyestalk-ablated	6	15.77±3.49	14.63 ± 2.16^{NS}

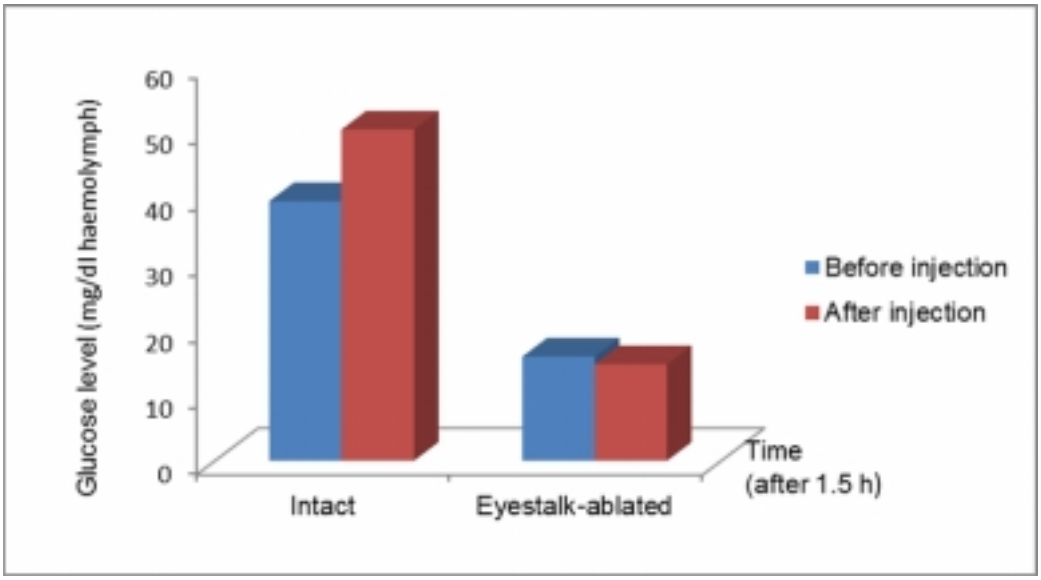


Fig 2: Rezults of adrenaline injection

Discussion

In the present study eyestalk ablation of the crabs leads to decrease in the glucose level in the haemolymph compared to the intact control animals, which maintain their own glucose level. In this experiment was noticed that the glucose level decreases drastically after 1.5 h in the haemolymph of eyestalk-ablated animals. Eeystalk extinpation is a classical operation of crustacean endocrinology, it removes the X-organ sinus gland complex, which is the source on an array hormones such as the crustacean Hyperglycemic Hormone (cHH). Removal of ey
estalks eliminates the cHH from circulation which results in significant decrease of glucose level in *Carcinus estuaries.* This shows clearly that the eyestalk sinus gland produce the crustacean hyperglycemic hormone that maintain the levels of haemolymph glucoce. The eyestalk extract injection increases the glucose level in intact and eyestalk-ablated animals too. The eyestalk extract injection into eyestalk-animals increased drastically the haemolymph glucose levels. This is due to tha lack of cHH , which is essential in maintaining the hyperglycemic condition. After the injection of eyestalk-exstract with cHH, the animals showed a sudden increase of the glucose level. The possible reason for this sudden increase might be the release of glucose from the hepatopancreas by the cHH corporation. Further more the eyestalk-ablation and the process of injection itself are stress factors on the crabs, which may have caused the secretion of glucose when supplied with cHH. In the intact animals since CHH was already mantaing the glucose level of hemolymph, the exess addition of eyestalk extract doesn't produse any significant effect.
Adrenaline injection increase drastically the haemolymph glucose level in the intact animals, while decreases slightly in eyestalk ablaled animals. When the animals are under the stress, the metabolic activities of the animals, increase rapidly and cause the hepatopancreas to release larger amounts of glucose in the haemolymph.

Conclusion:

1. The eyestalk-ablation in *Carcinus estuaris* results in marked decrease in concentration of blood-sugar because the removal of sinus gland eliminates the cHH from circulation and the extract injection leads to increase the glucose level.

2. Adrenaline, in doses from 1 to 1000, causes hyperglycemia in intact*Carcinus estuaries*
but injection into sinus-glandless crayfish shows either no rise in the concentration of blood-sugar, or a very slight decrease.
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3. The results of this study indicate the presence of an excitement hyperglycemia in these crustaceans, ensuing from release of diabetogenic hormone from the sinus glands. The effects of adrenaline in *Carcinusestuaries*may be due to its action on glandular tissue outside of the eyestalk, bringing about secretion of diabetogenic hormone468 L .H. KLEINHOLZ(in addition to its effect on the sinus glands) , or to its action on carbohydrate
storing tissue resulting in release of glucose.

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