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Initial Characterization of Hyperglycemic Hormone of the Freshwater Crab, *Barytelphusa cunicularis*

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Eyestalk extract of freshwater crab, *Barytelphusa cunicularis* contains a hyperglycemic hormone (HGH) that causes increase in blood sugar level within 90 min after injection. The dose response has a correlation with the protein concentration of the eyestalk extract. The HGH is fairly stable in weak alkaline conditions (*p*H 11.5), as compared to lower *p*H (4.5). Heat treatment at 100°C for 3 min. in aqueous solution, inactivated HGH activity. HGH is not dializable through cellophane bag, is inactivated by the action of several proteolytic enzymes (except papain) and is insoluble in several organic solvents. All above properties of eyestalk extract of *B. cunicularis* confirm the protenaceous nature of HGH.

Key Words: ***Barytelphausa cunicularis*, Hyperglycemic hormone, Characterization**

Introduction

There are many reports regarding purification and characterization of hyperglycemic hormone (HGH) of marine crustaceans (Kleinholz et al. 1950, 1967, Keller 1981, Martin et al. 1984); however, relatively little information is available about properties of HGH from freshwater crustaceans. This paper describes the initial characterization of HGH from the freshwater crab, *B. cunicularis*.

Material and Methods

Barytelphusa cunicularis collected from Kham river near Aurangabad (India) were kept in plastic containers containing dechlorinated tap water. Water was changed daily. Crabs were fed with pieces of earthworm twice in a week. Food was withdrawn 24 hr before the commencement of the experiment.

Five or more animals of either sex whose eyestalks had been removed 48 hr before, were used as control as well as experimental animals. The blood was drawn from the coxa of third walking leg of the crab with the help of hypodermic syringe. Blood sugar was estimated using phenol sulphuric acid method (Dubois et al. 1956), before and 90 min after the injection; thus each animal served as its own control (Kleinholz et al. 1967). The control animals were injected with 0.1ml of deionised water/saline/buffer depending upon experimental conditions while experimental animals received 0.1ml of test solution.

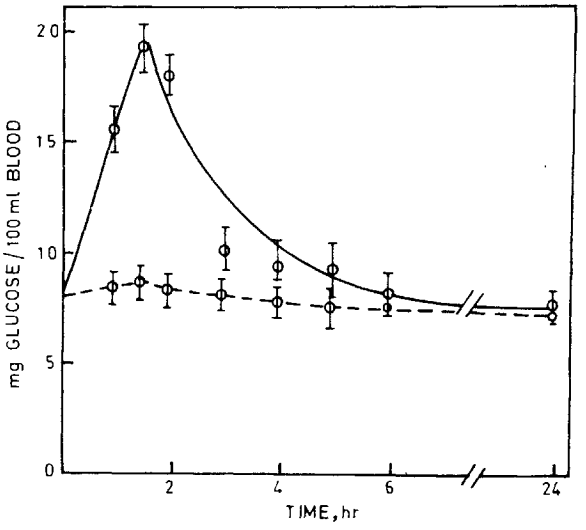
Acetone dried eyestalks (50) of *B. cunicularis* were extracted in crustacean saline (Van Harreveld 1936) and was used as hormonal source for characterization. Properties of solubility, stability at various *p*H and temperature (Kleinholz 1966), dialyzability and sensitivity to various proteolytic enzymes (Kleinholz et al. 1967) and time response (Keller & Andrew 1973) were studied. Protein was determined by coommassire dye method (Spector 1978).

Results

The maximum hyperglycemic response following the eyestalk injection was observed after 90 min of treatment. The hyperglycemia was maintained for a period of 6 hr (figure 1), after which blood sugar level was constant.

Increasing hyperglycemic response was observed with eyestalk extract protein (5 to 20 μg), though, further increase in protein concentration was ineffective. It appears that 20 μg/dose is optimum to induce maximum hyperglycemia (figure 2).

Insoluble fraction of eyestalks, extracted with acetone, ethanol and ether, induced an increase in blood



Fugure 1 Latency response curve of destalked *Barytelphusa cunicularis* to hyperglycemic hormone (20 .033g/0.1 ml). in eyestalk extract 0—0 Experimental injected with ES protein 0----0 Control injected with saline

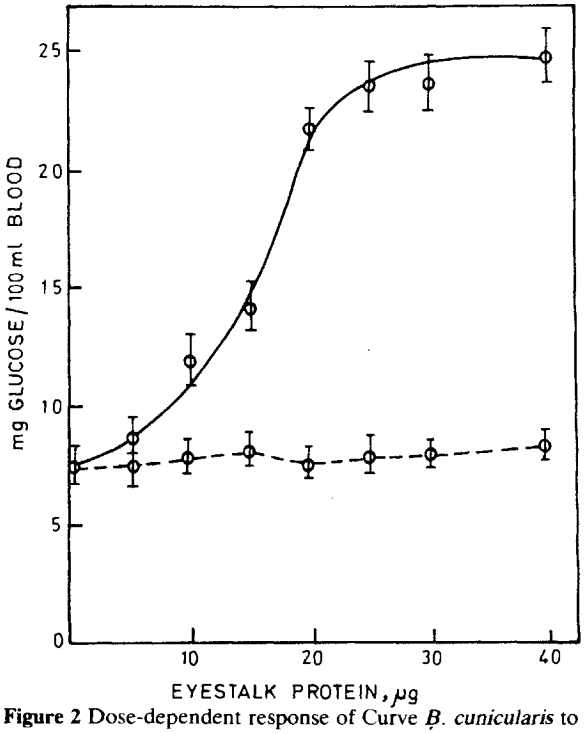


Figure 2 Dose-dependent response of Curve *B. cunicularis* to hyperglycemic hormone in eyetalk extract 0—0 Experimental injected with eyestalk extract; 0----0 Control injected with saline (0.1 ml)

sugar level, but not so when soluble fractions were injected (table 1).

Hyperglycemia was considerably low under acidic conditions but not at alkaline *p*H (table 2).

It is seen (table 3) that eyestalk extract, heated for 3 min, failed to increase blood sugar level compared to the unheated eyestalk extract.

Dialysis bag content induced fair hyperglycemic response, but not the dialysate or the buffer used (table 4).

The eyestalk extract treated with active enzymes such as trypsin and α-chymotrypsin failed to induce hyperglycemic response, but when treated with inacti-

Table 1 *Solubility of hyperglycemic hormone of* ***Barytelphusa cunicularis*** *in organic solvents*

Solvents	Fraction	Blood sugar (mg/100 ml + SD)	
		Before injection	After injection
Acetone	Soluble	8.6 ± 3.7	10.0 ± 3.5 NS
	Insoluble	8.1 ± 2.6	13.0 ± 3.8*
Ethanol	Soluble	7.9 ± 1.1	8.8 ± 4.0 NS
	Insoluble	8.1 ± 2.6	13.0 ± 2.4*
Ether	Soluble	8.4 ± 1.9	10.9 ± 4.0 NS
	Insoluble	8.4 ± 2.8	13.6 ± 2.9*
Saline eyestalk extract (control)		7.4 ± 3.5	24.0 ± 3.5*
Saline injected (Control)		7.5 ± 1.2	7.6 ± 1.4 NS

NS, Not significant; *P< 0.005

Table 2 *Stability of hyperglycemic hormone of* ***Barytelphusa cunicularis*** *at two* ***pH***

Incubating medium	*p*H	Blood sugar level (mg % ± SD)	
		Before injection	After injection
Eyestalk extract in acetate buffer (100 mM)	4.5	10.8 ± 1.8	11.3 ± 2.4*
Acetate buffer (100 mM)—Control	4.5	6.7 ± 0.6	7.2 ± 1.2 NS
Eyestalk extract in carbonate buffer (50 mM)	11.5	5.5 ± 1.3	23.2 ± 7.2*
Carbonate bicarbonate buffer (50 mM)—Control	11.5	7.1 ± 1.2	10.8 ± 1.2 NS
Saline eyestalk extract—Control	7.2	6.9 ± 0.4	22.3 ± 1.4*

NS = Not significant; * = P< 0.005

Table 3 *Thermostability of hyperglycemic hormone of* ***Barytelphusa cunicularis***

Treatment	Blood sugar level (mg % ± S.D.)	
	Before injection	After injection
Eyestalk extract heated at 100°C for 3 minutes	6.6±3.9	7.2±4.5 NS
Unheated eyestalk extract—Control	8.5±2.8	20.9±3.2*
Distilled water injected—Control	9.6±1.8	10.2±4.6 NS

NS, Not significant; * = $P < 0.005$

Table 4 *Effect of dialysis on hyperglycemic hormone of* ***B. cunicularis***

Conditions	Blood sugar level(mg % ±SD)	
	Before injection	After injection
Dialysis bag content	9.4±4.2	23.4±7.7*
Dialysate	11.0±3.0	10.8±2.0 NS
Tris-HCL (50 mM, *p*H 8.5) buffer —Control	10.2±2.8	11.9±2.2 NS
Undialysed eyestalk extract control	8.5±1.1	13.8±1.8*

NS, Not significant; *P < 0.005

vated enzymes or with active as well as inactive papain, induced hyperglycemia (table 5).

Discussion

In the freshwater crab, *B. cunicularis*, bilateral eyestalk removal caused hypoglycemia (Nagabhushanam & Diwan, 1974). Injection of eyestalk extract into destalked *B. cunicularis* induced rapid hyperglycemia, which peaked within 90 min after injection, though this rapidly declined to pre-treatment level within 6 hr. These results are consistent with those on other crustaceans viz. *Callinectes sapidus*, (Abramowitz et al. 1944) *Panulirus japonicus* and *Panulirus peniculatus* (Scheer & Scheer, 1951), *Scylla serrata* (Menon & Sivadas 1967) and *Uca pugilator* (Keller & Andrew 1973). It seems, that, in crustaceans, the circulating hormone is inactivated within 6 hr and large amount of released sugar is again adsorbed by the respective tissue at a rapid rate. Similar results were obtained by Kleinholz et al. (1967) in *Pandalus borealis* and by Kleinholz and Keller (1973) in *Cancer magister*. They observed the hyperglycemic peak 2 hr after the injection. Skorkowski et al. (1977) observed peak hyperglycemic level in the shrimp, *Crangon crangon* 2 hr after injection and then a decline.

The minimum dose required to cause maximum hyperglycemia in *B. cunicularis* is 20 μg of the eyestalk proteins. Kleinholz and Keller (1973) reported 100% increase in blood sugar over the preinjected level with 10 μg of eyestalk proteins from *Cancer* and *Uca* and with 30 μg from *Procambarus clarkii*, whereas Skorkowski et al. (1977) recorded that 20 μg of eyestalk protein was needed for maximum hyperglycemia in *C. crangon*.

The present study reveals that hyperglycemic hormone of *B. cunicularis* is insoluble in organic solvents such as acetone, ethanol and ether and is thermolabile. At high temperature, the hormone must be loosing its original structure. It was observed to be fairly stable in alkaline *p*H though its activity was markedly reduced at acidic *p*H. This suggests that high H^+ concentration affects native molecular structure, thus reducing its activity. Hyperglycemic hormone of *B. cunicularis* is non-dialysable through cellophane tube. When sensi-

Table 5 *Sensitivity of hyperglycemic hormone of* ***Barytelphusa cunicularis*** *to various proteolytic enzymes*

Enzyme	Buffer medium			Blood sugar level (mg % ± SD)	
	Buffer	Molarity	*p*H	Before injection	After injection
Trypsin	Tris HCl	100 mM	8.0	9.6±2.8	7.7±2.7 NS
Boiled trypsin	-do-	-do-	8.0	8.4±0.9	16.8±4.3*
Chymotrypsin	-do-	-do-	8.0	7.6±4.1	7.2±6.3
Boiled "	-do-	-do-	8.0	9.0±3.7	19.3±16.1*
Papain	-do-	-do-	6.5	8.8±3.9	18.8±3.2*
Boiled papain	-do-	-do-	6.5	8.0±0.6	19.4±2.3*
Untreated saline eyestalk extract	—	—	7.2	7.8±1.8	17.8±2.3*

NS, Not significant; *P < 0.005

tivity to various proteolytic enzymes was studied it was seen that this eyestalk factor is inactivated by enzymes such as trypsin and α-chymotrypsin. However, it is not sensitive to the enzyme papain. These findings are in agreement with those of Kleinholz et al. (1967).

Insolubility in various organic solvents and thermolability and sensitivity to various proteolytic enzymes leads one to assume that hyperglycemic hormone from eyestalks of *B. cunicularis* may be a polypeptide.

Presence of hypoglycemic factor in the eyestalks has been reported by Rangnekar et al. (1961) in the crab, *Barytelphusa jaquemontii*. Deshmukh and Rangnekar (1973) reported the presence of both hypoglycemic and hyperglycemic factors in the eyestalk of the crab, *Scylla serrata*. It suggests the possibility of a dual control mechanism analogus to that found in vertebrates. Pezalla et al. (1978) observed the hyperglycemic factor in the central nervous system of *Limulus polyphemus* causing hyperglycema in freshwater crayfish, *Orconectes immunis*. This factor, inactivated by hydrogen peroxide, pepsin and protease, was not affected by trypsin and brief boiling. In order to understand endocrine control of carbohydrate metabolism in Crustacea, hypo-and hyperglycemic factors outside eyestalks must also be studied.

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