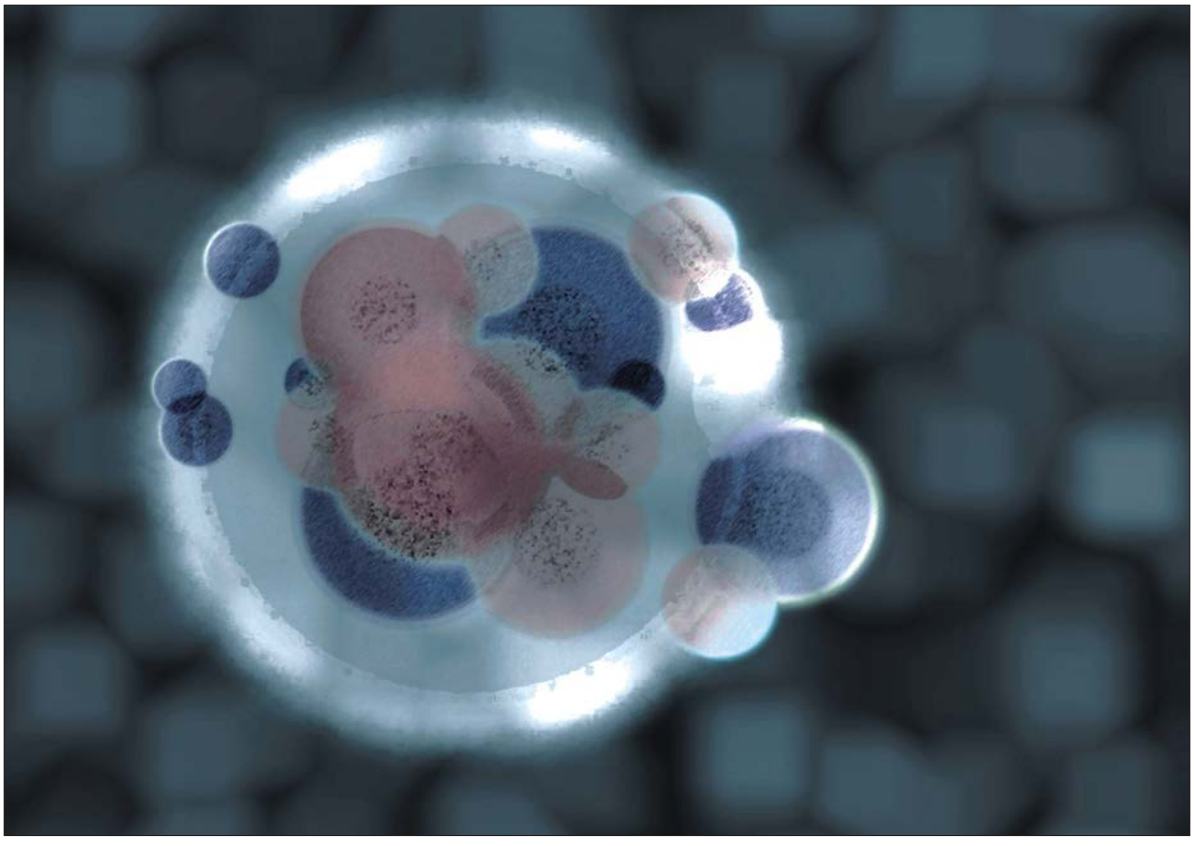

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Differential Mode of Action of Hyperglycemic Hormones Obtained from Crab (Crustacea) and Millipede (Myriapoda) in the Crab, *Oziotelphusa senex senex*

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Abstract: Injection of hyperglycemic hormones obtained from crab (*Oziotelphusa*) and millipede (*Spirostreptus*) showed different effects on tissue phosphorylase activity and levels of total carbohydrates and glycogen in the crab, *Oziotelphusa senex senex*. Hyperglycemic hormones obtained from both sources caused significant elevation of hemolymph glucose level in crab in a dose-dependent manner. The crab hyperglycemic hormone induces phosphorolysis of glycogen, whereas the phosphorylase system is not affected by millipede hyperglycemic hormone. The crab hyperglycemic hormone decreased carbohydrate levels in hepatopancreas and muscle, whereas the tissue carbohydrate levels increased after millipede hyperglycemic hormone injection. Administration of millipede hyperglycemic hormone led to significant decrease in tissue lipids, suggesting the involvement of lipids in causation of hyperglycemia. Greater incorporation of labeled acetate-1-^{14}C into the carbohydrate fractions after the injection of millipede hyperglycemic hormone supports the above mode of action. The results demonstrate the existence of at least two parallel mechanisms of hormonal regulation of glycemia in arthropods and point to inter-specific action of arthropod hyperglycemic hormones.

Key words: Hyperglycemic hormone, *Oziotelphusa*, *Spirostreptus*, hyperglycemia, phosphorylase, tissue glycogen and total carbohydrates

INTRODUCTION

The role of the neuro-endocrine system in the regulation of metabolism has been explored in myriapoda only to a limited extent. The available information on myriapod neuroendocrinology pertains mostly to descriptions of the neurosecretory cell groups (Gabe, 1954; Prabhu, 1962, 1964). The existence of a hyperglycemic factor in the cerebral ganglia of the millipede, *Spirostreptus*, has been demonstrated (Satyam and Ramamurthi, 1978). Satyam *et al.* (1982) suggested that the tissue lipids are the source for hyperglycemia in millipedes.

Observations that eyestalk factors may regulate hemolymph glucose concentration in crustaceans were made several decades ago by Abramowitz *et al.* (1944). They described a diabetogenic factor found in aqueous extracts of the crab *Uca pugilator* that when injected into the blue crab *Callinectes sapidus*, produced an intense hyperglycemia in a very short time. They also localized this diabetogenic activity in the neurohaemal sinus gland in the eyestalk. The hormone(s) responsible for this hyperglycemic effect is commonly referred to as the Crustacean Hyperglycemic Hormone (CHH). This hormone is produced by the neurosecretory cells in the X-organ region of the eyestalk, transported to, stored in and then released by the sinus gland into the hemolymph. Upon injection of CHH a decrease in tissue glycogen is observed mainly in the hepatopancreas and muscle of crustaceans. The injection of CHH rapidly inactivates the glycogen synthase and probably activates the phosphorylase in a time course comparable to the hyperglycemia (Keller, 1965; Sedlmeier, 1982, 1987; Sedlmeier and Keller, 1981). This action is very similar to glucagon, a hormone released by the pancreas of the vertebrates.

The amino acid sequences of CHHs have been determined by Edman degradation and in some cases, also by cloning of the precursor cDNA (for reviews see Keller, 1992; Chang, 1993; DeKleign and Van Herp, 1995). The CHH has also been purified from the experimental model of this present study, the fresh water crab *Oziotelphusa senex senex* (Reddy and Reddy, 2006). But very little is known about the interspecific action of the hyperglycemic hormones (Leuven *et al*., 1982).

A programme for study of interspecific action of several invertebrate hormones has been under taken in this laboratory. The first report describes the effect of

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crustacean eyestalk extract on carbohydrate levels in the tissues of a scorpion, *Heterometrus fulvipes* (Reddy and Ramamurthi, 1980). Only brief accounts of work have been published to date (Reddy and Ramamurthi, 1981, 1982; Reddy *et al*., 1982a-c). The present report is a part of that program, examines the effect of hyperglycemic hormones obtained from a millipede (MHH) and crab (CHH) on the hemolymph sugar levels and on identification of the hyperglycemia-source-material in the fresh water field crab, *Oziotelphusa senex senex* Fabricius.

MATERIALS AND METHODS

Crabs collection and acclimatization: Adult, 25-30 g crabs (*Oziotelphusa senex senex*) were collected from local rice fields and acclimated to laboratory conditions for 10 days in glass aquaria (water temperature 27±1°C; photoperiod 12 light: 12 dark) at Department of Biotechnology, S.V. University, Tirupati. The animals were fed with frog muscle daily *ad libitum*. Only uninjured, intact crabs were used in the present study. Since feeding affects glycemia (Parvathy, 1971), crabs were starved for at least 2 days before experimentation. To minimize possible effects of the molt cycle on glycemia (Reddy, 1990), only intermolt (Stage C_4) crabs were used in the present study. To reduce the possible effects of reproductive condition on glycemia (Ramamurthi and Verabhadrachari, 1975), only male crabs were utilized. Owing to the existence of a diurnal rhythm of glycemia in crabs (Reddy *et al*., 1986), all the experiments were carried out between 10.00 AM and 12.00 Noon. Since temperature noticeably affects the blood glucose level in crustaceans (Dean and Vernberg, 1965), all experiments were conducted at controlled temperatures of 27±1°C.

Hyperglycemic hormone extraction: Crustacean hyperglycemic hormone was isolated from the sinus glands of the crab *Oziotelphusa senex senex*. Sinus glands were dissected from freshly excised eyestalks from intermolt crabs. Millipede (*Spirostretus asthenes*) hyperglycemic hormone (MHH) was isolated from the cerebral ganglion. Freshly collected glands were transferred to a homogenizer, disrupted in 0.1 N HCl and heated for 5.0 min at 80°C. The homogenates were centrifuged at 16,000 X g for 5.0 min. The supernatant was neutralized with 100 μL of 4.0 M sodium acetate and subjected to reverse phase high performance liquid chromatography using a column of C18 sep-pak. The materials were eluted with a linear gradient of acetonitrile from 0 to 65% in 0.05% trifluoroacetic acid at a flow rate of 1.0 mL min^{-1}. The elution was monitored by UV absorbance at both 225 and 280 nm. Fractions were collected peak by peak at 225 nm and subjected to a bioassay for CHH, extremely high activity fraction was used as hyperglycemic hormone.

The crabs were divided into 4 groups of ten animals each. The crabs in the first group received no treatment and served as normal crabs; the crabs in the second group were injected with 20 μL crustacean ringer solution (Van Harreveld, 1936) and served as controls; third and fourth group animals were injected with 20 μL of CHH and MHH (2 eyestalk equivalents of CHH and one cerebral ganglion equivalent of MHH), respectively. Based on our preliminary experiments (Fig. 1 and 2), the crabs were sacrificed 2 h after injection and hemolymph, hepatopancreas and muscle tissues from propodus of chela were isolated and used for estimation of glycogen, total carbohydrates and phosphorylase activity.

Biochemical analysis: The tissues were analyzed for the following biochemical estimations.

Estimate of hemolymph glucose level: For measurement of glucose, 100 μL of hemolymph was mixed with 300 μL of 95% ethanol in a 1.5 mL micro tube. The precipitated proteins were pelleted (4°C, 14,000 X g, 10.0 min) and to the samples (100 μL), a mixture of glucose enzyme reagent (glucose-6-phosphate dehydrogenase and NADP) and color reagents (phenazine methosulfate and iodonitrotetrazolium chloride (200 μL) was added (kit from Sigma). After 30.0 min. the optical density was read using an ELISA reader at 490 nm. A standard curve constructed using glucose standards was used for glucose quantification.

Estimate of tissue glycogen and total carbohydrate contents: The tissue total carbohydrate content (TCHO) (in hepatopancreas and chela muscle) was estimated in trichloroacetic acid (TCA) supernatant (5% W/V) and glycogen in the ethanolic precipitate of TCA supernatants (Carroll *et al*., 1956).

Assay of tissue glycogen phosphorylase: Tissue phosphorylase activity was assayed in the direction of glycogen synthesis (Cori *et al*., 1955) by the colorimetric determination of inorganic phosphate released from glucose-1-phosphate. Tissue homogenates (5% W/V) were prepared in an aqueous medium containing 0.037 M ethylene diamine tetra acetic acid and 0.1 M sodium fluoride, pH 6.5. The homogenate was centrifuged for 10 min at 2,500 rpm and the supernatant was diluted four times with cysteine (0.03 M), β-glycerophosphate (0.015 M) buffer, pH 6.5. The diluted enzyme (0.5 mL) was added to 0.2 mL of 2% glycogen and incubated for 20 min

at 35°C. The reaction was initiated by the addition of 0.2 mL of 0.016 M glucose-1-phosphate to one tube (phosphorylase a), 0.2 mL of 0.016 M glucose-1-phosphate and 0.004 M adenosine-5-monophosphate to the other (phosphorylase ab). After incubation for 15 min for phosphorylase ab (total) and 30 min for phosphorylase a (active), the reaction was arrested by the addition of 5.0 mL of 5 N sulphuric acid. The inorganic phosphate liberated was estimated by the method of Fiske and Subbarao (1925).

Estimate of protein content: The protein content in the enzyme source was estimated following the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Radiometric study: A batch of twelve crabs was injected with 1 μCi of labeled acetate-1-^{14}C and the label was allowed to accumulate in the body for 24 h. One set of six crabs was used as controls and the remaining six crabs received an injection of MHH (one cerebral ganglion equivalent). Based on earlier time-course experiment, the tissues were isolated 2 h after MHH injection. For radiometry of proteins, carbohydrates, lipids and amino acids of hemolymph and hepatopancreas, the tissues were prepared according to the procedure given by Vrba and Cannon (1970), Vrba and Winter (1972, 1973) and Ramamurthi *et al.* (1981). The radioactivity of samples was measured in LKB 1217 Rackbeta liquid scintillation counter. The levels of proteins (Lowry *et al.*, 1951), total carbohydrates (Carrol *et al.*, 1956), total lipids (Folch *et al.*, 1957) and amino acids (Moore and Stein, 1968) were also estimated in the tissues of both sets.

Analysis of results: The data were analyzed using SPSS 10.0 version for significant differences by analysis of variance (ANOVA). If the F was found significant, it was followed by SNK test. Results are given as means±SD; F-values (F), degrees of freedom (df) and significance level (p).

RESULTS

Injection of either CHH or MHH increased the hemolymph glucose level in *Oziotelphusa senex senex* in a dose-dependant manner (for CHH: F = 757.25; df = 7, 72; p<0.0001; for MHH: F = 470.78; df = 7, 72; p<0.0001) (Fig. 1). A time-course for CHH or MHH induced hyperglycemia is shown in Fig. 2. The hemolymph glucose level increased significantly within 30 min of injection, reached a peak at 2 h, then declined, reaching basal levels at 6 h (for CHH: F = 108.79; df = 5, 54; p<0.0001; for MHH: F = 192.42; df = 5, 54; p<0.0001) (Fig. 2).

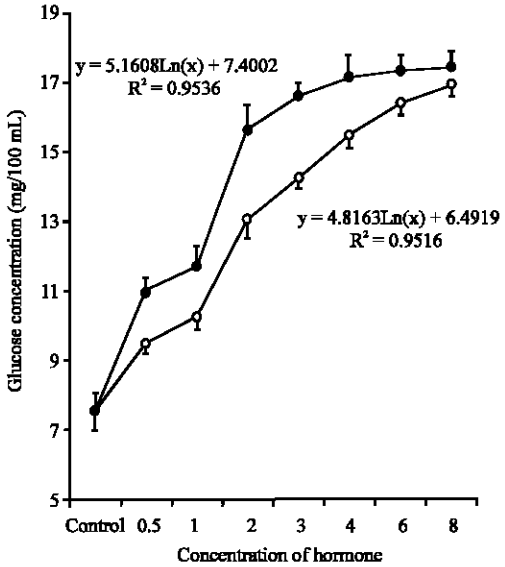

Fig. 1: Dose-dependent effect of CHH (open circles) or MHH (closed circles) on the hemolymph glucose levels in crab *Oziotelphusa senex senex*. Two hours after injection of hormone at the doses indicated (eyestalk equivalent or cerebral ganglia equivalent), hemolymph was withdrawn from crabs for glucose determination. Each point represents a mean±SD (n = 10)

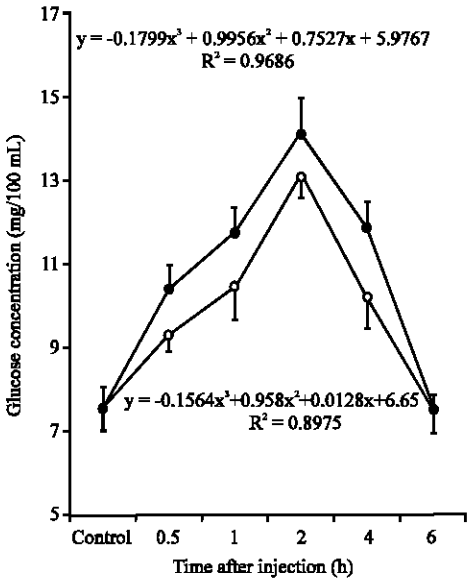

Fig. 2: Time course of CHH (open circles) or MHH (closed circles) induced hyperglycemia in crab *Oziotelphusa senex senex*. After injection of hormone (two eyestalks or cerebral ganglia equivalent/crab), hemolymph was withdrawn from animals at the time points indicated for glucose determination. Each point represents a mean±SD (n = 10)

Table 1: Effect of injection of CHH or MHH on total carbohydrates and glycogen levels in the tissues of crab *Oziotelphusa senex senex*

Component	Tissue	Normal	Control (Ringer injected)	CHH	MHH
Total carbohydrates	Hemolymph	75.62±9.87	78.08±8.77^{NS}	110.95±11.51*	114.53±9.88*
			(+ 3.27)	(+ 46.72)	(+ 51.46)
	Hepatopancreas	30.21±4.09	31.61±6.43^{NS}	20.09±4.11*	50.44±7.31*
			(+ 4.63)	(- 33.50)	(+ 66.97)
	Muscle	7.54±0.55	7.49±0.61^{NS}	5.01±0.61*	9.87±0.91*
			(- 0.66)	(- 33.55)	(+ 30.90)
Glycogen	Hepatopancreas	4.67±0.41	4.90±0.52^{NS}	1.88±0.19*	4.99±0.67^{NS}
			(+ 4.93)	(- 59.74)	(+ 6.85)
	Muscle	1.73±0.09	1.81±0.11^{NS}	0.97±0.07*	1.75^{NS}±0.12^{NS}
			(+ 4.62)	(- 43.93)	(+ 1.16)

Values are mean (mg of glucose/100 mL of hemolymph; mg of glucose/g wet wt. of tissue)±SD of 10 individuals. Values in parentheses are % change over normal; *p<0.001; NS = Not Significant

Table 2: Effect of injection of CHH or MHH on the activity levels of active and total phosphorylase in hepatopancreas and muscle of crab *Oziotelphusa senex senex*

Tissue	Enzyme	Normal	Control (Ringer injected)	CHH	MHH
Hepatopancreas	Active	2.27±0.33	2.19±0.41^{NS}	3.69±0.42*	2.29±0.37^{NS}
			(- 3.52)	(+ 62.56)	(+ 0.88)
	Total	4.56±0.64	4.31±0.51^{NS}	5.69±0.67**	4.64±0.60^{NS}
			(- 5.48)	(+ 24.78)	(+ 1.75)
	Active/total	0.49	0.51	0.65	0.49
			(+ 4.08)	(+ 32.65)	-
Muscle	Active	4.03±0.44	4.05±0.50^{NS}	6.27±0.73*	4.09±0.53^{NS}
			(+ 0.50)	(+ 55.58)	(+ 1.49)
	Total	7.31±0.77	7.35±0.69^{NS}	8.94±0.83*	7.55±0.88^{NS}
			(+ 0.55)	(+ 22.29)	(+ 3.28)
	Active/total	0.55	0.55	0.70	0.54
			-	(+ 27.28)	(- 1.82)

Values are mean (μ moles of inorganic phosphate released/mg protein/h)±SD of 10 individuals. Values in parentheses are % change over normal; *p<0.001; **p<0.01; NS = Not Significant

The data (Table 1 and 2) also reveal the existence of a fundamental difference in the mode of action of these hyperglycemic principles on the regulation of levels of tissue total carbohydrate, glycogen and activity levels of phosphorylase. CHH caused a significant (p<0.001) decrease of the total carbohydrate level of hepatopancreas (-33.5%) and muscle (-33.6%), whereas MHH injection resulted in a significant (p<0.001) increase tissue total carbohydrate level (+66.9% in hepatopancreas; +30.9% in muscle). Glycogen content decreased significantly (p<0.001) in crab hepatopancreas and muscle after CHH injection, while MHH injection resulted in an insignificant change in tissue glycogen level (Table 1).

The apparent difference in the mode of action of two hyperglycemic hormones is further illustrated by their actions on tissue phosphorylase activity. Injection of CHH elevated both active (a) and total (ab) tissue phosphorylase activity levels. Also CHH elevated the ratio of active to total phosphorylase by 32.7% in hepatopancreas and 27.3% in muscle over control. By contrast, MHH has no effect on this enzyme system (Table 2). Yet injection of both these hormones led to development of significant hyperglycemia in the crab.

DISCUSSION

Hyperglycemia observed in the present study, in the crab, *Oziotelphusa senex senex* with CHH and MHH indicates that the crab tissues show good responsiveness to both hormones. However the data on the mode of action of these hormones appear to suggest that these hormones have different *modi operandi* in causation of hyperglycemia. An increased phosphorylase activity and a decrease in glycogen and total carbohydrate levels in hepatopancreas and muscle of crab, followed by hyperglycemia after injection of CHH indicates glycogenolysis and mobilization of sugar molecules from tissues to hemolymph. This is in agreement with the previous reports on different decapod crustaceans (Keller, 1992). Earlier, Hohnke and Scheer (1970) suggested that the primary role of CHH is not to elevate hemolymph sugar level but to elevate intra cellular glucose levels through the degradation of glycogen by activating the enzyme phosphorylase. Reddy (1990) observed inter-conversion of inactive phosphorylase to active phosphorylase, which results in glycogenolysis and release of glucose from tissues of crab *Oziotelphusa senex senex* after eyestalk extract

injection. These glucose molecules leak into the hemolymph resulting in hyperglycemia (Keller and Andrew, 1973; Telford, 1975; Kishori *et al*., 2001; Reddy and Kishori, 2001). It is of interest to note that insects possess in their brain a hyperglycemic hormone, the action of which appears to be similar to CHH in its mode of action on the fat body carbohydrates and phosphorylase (Steele, 1961, 1963).

The MHH does not activate the phosphorylase system; it does, however, elevate the total carbohydrate content in the tissues and also hyperglycemia. In view of its significant accumulation under the influence of MHH, the carbohydrate content of tissues of the crab may not be implicated as an important causal source of hyperglycemia. The effect of MHH in the crab is similar to its effect in its `native' (millipede) milieu (Satyam *et al*., 1982). Satyam *et al*. (1982) reported a significant ($p<0.001$) decrease (-72%) in fat body lipid content and hyperglycemia in the millipede *Spirostreptus asthenes* after injection its own cerebral ganglia extract. Decreased uptake of acetate-1-^{14}C into the total lipid fraction of the fat body of *Spirosterptus* was also reported after the injection of cerebral ganglia extract (Satyam, 1976). Satyam (1976) observed increased incorporation of labeled acetate into the carbohydrate fraction of fat body after cerebral ganglion extract injection. Based on these observations one may conclude tentatively that under the influence of MHH, the lipids of the fat body are converted to carbohydrates and this neo-carbohydrate is responsible for the hyperglycemia (Table 3).

In view of its significant decrease under the influence of MHH, the total lipid content of hepatopancreas may be implicated as an important causal source for hyperglycemia in the crab resulting under the same aegis (Table 4). This is further confirmed by the elevation in the uptake of label from radio-acetate into hemolymph carbohydrate fractions. Similar type of hyperglycemic response was reported in arachnids (Raghavaiah *et al*., 1977, 1978). In the scorpion, *Heterometrus fulvipes* the action of hyperglycemic hormone seems to involve the mobilization of tissue lipids for causation of hyperglycemia (Raghavaiah *et al*., 1977).

Research progress in endocrinology of arthropods has historically lagged behind studies using insect systems. Recently, an increase in crustacean endocrine research has occurred because of interest in aquacultural activities. As is true of most biological systems the more that a system is investigated the more complex it appears. Certainly the hyperglycemic hormones of arthropods are more diverge and operate in different ways. One of the most interesting outcomes of our study is the analogy observed between insects and crustaceans with regard to the mode of action of hyperglycemic hormone and their divergence from arachnids and myriapods. In addition, a factor that regulates ion transport in a locust (Audsley *et al*., 1992; Meredith *et al*., 1995) and a peptide that co-migrates with a spider toxin (Gasparini *et al*., 1994), both appear to be members of the CHH family. This suggests that members of CHH family of neuropeptides appear to be present in different classes of arthropoda

Table 3: Effect of MHH injection on the incorporation of labelled acetate-1-^{14}C into various organic fractions in hepatopancreas and hemolymph of crab, *Oziotelphusa senex senex*

	Hepatopancreas		Hemolymph	
Fraction	Control	MHH	Control	MHH
Total carbohydrates	4.28±0.14	10.97±0.19* (+ 156.31)	1.77±0.17	4.79±0.31* (+ 170.62)
Free amino acids	1.25±0.09	1.27±0.17^{NS} (+ 1.60)	1.64±0.12	1.62±0.14^{NS} (+ 1.22)
Total proteins	2.81±0.73	2.79±0.91^{NS} (- 0.71)	1.09±0.14	1.11±0.13^{NS} (+ 1.83)
Total lipids	34.66±4.41	16.26±3.47* (- 53.09)	12.51±3.11	9.01±3.15* (- 27.98)

Values are mean (cpm mg^{-1} organic fractions)±SD of six individuals. Values in parentheses are % change over control. *$p<0.001$; NS = Not Significant

Table 4: Effect of MHH injection on the levels of organic constituents in the hemolymph and hepatopancreas of crab, *Oziotelphusa senex senex*

	Hepatopancreas		Hemolymph	
Fraction	Control	MHH	Control	MHH
Total carbohydrates	31.4±1.79	50.97±2.94* (+ 62.27)	73.88±4.61	118.66±9.64* (+ 60.61)
Free amino acids	18.22±4.51	28.94±8.51* (+ 58.84)	51.01±6.54	69.55±7.99* (+ 36.35)
Total proteins	135.41±2.56	110.51±14.37** (- 18.39)	34.09±5.94	28.44±6.18** (- 16.57)
Total lipids	146.95±11.97	54.27±8.61* (- 63.07)	94.73±7.47	56.41±5.09* (- 40.45)

Values are mean (mg g^{-1} tissue; mg/100 mL hemolymph)±SD of 6 individuals. Values in parentheses are % change over control. *$p<0.001$; **$p<0.01$

and can have different functions (acting as a hyperglycemic hormone in crustacean, as a ion-regulating hormone in insecta and as a toxic peptide in arachnida). This example illustrates the amazing economy of nature-a single peptide hormone that can mediate different functions in different classes of arthropods.

The data of the present study clearly indicate the existence of two parallel mechanisms of hormonal regulation of glycemia in arthropods-hyperglycemic hormones obtained from crustaceans and insects induce glycogenolysis; whereas hyperglycemic hormones obtained from arachnids and myriapods acts through neo-glucogenesis. However, the MHH, when introduced into the crab, acts through the same mechanism as it does in millipede (Satyam *et al*., 1982). Although the crab has hyperglycemic hormone of a different nature, its tissues do respond with equal facility to MHH.

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REFERENCES

Abramowitz, A., N. Hisaw and D. Papendrea, 1944. The occurrence of a diabetogenic factor in the eyestalks of crustaceans. Biol. Bull., 86: 1-4.

Audsley, N., C. McIntosh and J.E. Phillips, 1992. Isolation of a neuropeptide from locust copus cardiacum which influences ileal transport. J. Exp. Biol., 173: 261-274.

Carroll, N.V., N. Longley and J.H. Roe, 1956. Glycogen determination in liver and muscle by use of anthrone reagent. J. Biol. Chem., 220: 583-593.

Chang, E.S., 1993. Comparative endocrinology of molting and reproduction: Insects and crustaceans. Ann. Rev. Entomol., 38: 161-180.

Cori, G.T., B. Illingworth and G. Keller, 1955. Muscle Phosphorylase. In: Methods in Enzymology. Colowick, S.P. and N.O. Kaplan (Eds.). Academic Press, New York, pp: 200-215.

Dean, J.M. and F.J. Vernberg, 1965. Variation in blood glucose level of crustacea. Comp. Biochem. Physiol., 14: 29-34.

DeKleign, D.P.V. and F. Van Herp, 1995. Molecular biology of neurohormonal precursors in the eyestalks of crustacean. Comp. Biochem. Pysiol., 112: 573-579.

Fiske, C.H. and Y. Subbarao, 1925. The colorimetric determination of phosphorous. J. Biol. Chem., 66: 375-400.

Folch, J.M., M.P. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 266: 297-309.

Gabe, M., 1954. Emplacement et connexions des cellules neurosecretrices chez quelques Diplopodes. C.R. Acad. Sci. Paris., 239: 828-832.

Gasparini, S., N. Kiyaatkin, P. Drevet, J.C. Boulain, F. Tacnet, P. Ripoche, E. Forest, E. Grishin and A. Menez, 1994. The low molecular weight protein which co-purifies with α-latrotoxin is structurally related to crustacean hyperglycemic hormones. J. Biol. Chem., 269: 19803-19809.

Hohnke, L. and B.T. Scheer, 1970. Carbohydrate Metabolism in Crustaceans. In: Chemical Zoology. Florkin, M. and B.T. Scheer (Eds.). Academic Press, New York, pp: 147-166.

Keller, R., 1965. Uber eine hormonale kontrolle des polysaccharirstoffwechsels beim fluss krebs *Cambarus affinis*. Z. Vergl. Physiol., 51: 49-59.

Keller, R. and E.M. Andrew, 1973. The site of action of the crustacean hyperglycemic hormone. Gen. Comp. Endocrinol., 20: 572-579.

Keller, R., 1992. Crustacean neuropeptides: Structures, functions and comparative aspects. Experientia, 48: 439-448.

Kishori, B., B. Premasheela, R. Ramamurthi and P.S. Reddy, 2001. Evidence for hyperglycemic effect of methionine-enkephalins in the prawns, *Peneaus indicus* and *Metapenaeus monoceres*. Gen. Comp. Endocrinol., 123: 90-99.

Leuven, R.S.E.W., P.P. Jaros, F. Van Herp and R. Keller, 1982. Species or group specificity in biological and immunological studies of crustacean hyperglycemic hormone. Gen. Comp. Endocrinol., 46: 288-296.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Meredith, J., N. Cheng, D. Littleford, M. Ring, N. Audsley, H. Brock and J.E. Phillips, 1995. Amino acid sequence of locust Ion Transport Peptide (ITP). FASEB J., 9: A355.

Moore, S. and W.H. Stein, 1968. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 243: 6281-6283.

Parvathy, K., 1971. Blood sugar metabolism during molting in the isopod crustacean, *Ligia exotica*. Mar. Biol., 9: 323-327.

Prabhu, V.K.K., 1962. Neurosecretory system of *Jonespeltis splendidus*. Mem. Soc. Endocrinol., 12: 417-419.

Prabhu, V.K.K., 1964. Regeneration of connective bodies of the neurosecretory system of *Jonespeltis splendidus*. Indian J. Exp. Biol., 2: 5-8.

Raghavaiah, K., V.S.R. Murthy, R. Ramamurthi, P. Satyam and V. Chandrasekharam, 1977. The effect of epinephrine and the hyperglycemic factor of scorpion cephalothoracic nerve mass on the phosphorylase activity of hepatopancreas of the scorpion *Heterometrus fulvipes* (Koch). Experientia., 33: 690-691.

Raghavaiah, K., K. Satyanarayana, R. Ramamurthi and V. Chandrasekharam, 1978. On the hyperglycemic principle of the cephalothoracic ganglionic mass of the South Indian scorpion *Heterometrus fulvipes*. Indian J. Exp. Biol., 16: 944-946.

Ramamurthi, R. and V. Veerabhadrachari, 1975. Carbohydrate levels in freshwater crab *Paratelphusa hydrodromous* (Herbst). Indian J. Exp. Biol., 13: 76-78.

Ramamurthi, R., K. Raghavaiah, V.S.R. Murthy, V. Chandrasekharam and B.T. Scheer, 1981. Neuroendocrine control of nitrogen metabolism in the Indian field crab, *Oziotelphusa senex senex* (Fabricius). II. Incorporation of label from radio-amino acids into the tissue organic fractions. J. Reprod. Biol. Comp. Endocrinol., 1: 75-79.

Reddy, P.S. and R. Ramamurthi, 1980. Effect of crustacean eyestalk extract on the carbohydrate levels in the South Indian scorpion *Heterometrus fulvipes* (Koch). Experientia, 36: 318-319.

Reddy, P.S. and R. Ramamurthi, 1981. Interspecific effects of crustacean hyperglycemic hormone. Indian J. Exp. Biol., 19: 472-474.

Reddy, P.S. and R. Ramamurthi, 1982. The effect *in vivo* of crustacean eyestalk extract on the aspects of carbohydrate metabolism in the slug, *Laevicaulis alte*. Arch. Internat. Physiol. Biochim., 90: 9-14.

Reddy, P.S., A. Bhagyalakshmi and R. Ramamurthi, 1982a. Lack of group specificity of arthropod hyperglycemic hormone. J. Reprod. Biol. Comp. Endocrinol., 2: 69-72.

Reddy, P.S., A. Bhagyalakshmi, R. Ramamurthi and V. Chandrasekharam, 1982b. Hyperglycemic activity of crab and scorpion hormones in grass hopper (*Poecilocerus pictus*). Experientia., 38: 811-812.

Reddy, P.S., A. Bhagyalakshmi and R. Ramamurthi, 1982c. Evaluation of participation of glucose in elevation of glycemia of scorpion *(Heterometrus fulvipes)* caused by the hyperglycemic principles of the scorpion and crab. Experientia, 39: 1354-1355.

Reddy, P.S., A. Bhagyalakshmi and R. Ramamurthi, 1986. Diurnal rhythm of hemolymph sugar and hyperglycemic activity of eyestalk extract in the freshwater rice field crab, *Oziotelphusa senex senex*. Arch. Internat. Physiol. Biochim., 9: 193-195.

Reddy, P.S., 1990. Neuroendocrine control of metabolism in the fresh water crab *Oziotelphusa senex senex*. J. Crust. Biol., 10: 595-607.

Reddy, P.S. and B. Kishori, 2001. Methionine-enkephalin induces hyperglycemia through eyestalk hormone(s) in the esturine crab *Scylla serrata*. Biol. Bull., 201: 17-25.

Reddy, P.R. and P.S. Reddy, 2006. Isolation of peptide hormones with pleotropic activities in fresh water crab *Oziotelphusa senex senex*. Aquaculture, 259: 424-431.

Satyam, P., 1976. Biochemical studies on myriapods: Inorganic and organic composition of hemolymph and fat body of the millipede, *Spirostreptus asthenes*. Doctoral Dissertation, S.V. University, Tiurpati, India.

Satyam, P. and R. Ramamurthi, 1978. Neuroendocrine control of metabolism in millipede *Spirostreptus asthenes*. Abh. Verh. Naturwiss., 21: 325-327.

Satyam, P., V. Chandrasekharam and R. Ramamurthi, 1982. Hyperglycemic effect of cerebral ganglia of the millipede *Spirostreptus asthenes*. Indian J. Exp. Biol., 20: 913-914.

Sedlmeier, D. and R. Keller, 1981. The mode of action of the crustacean neurosecretory hyperglycemic hormone. I. Involvement of cyclic nucleotides. Gen. Comp. Endocrinol., 45: 82-90.

Sedlmeier, D., 1982. The mode of action of the crustacean neurosecretory hyperglycemic hormone (CHH). II. Involvement of glycogen synthase. Gen. Comp. Endocrinol., 47: 426-432.

Sedlmeier, D., 1987. The role of hepatopancreatic glycogen in the action of the crustacean hyperglycemic hormone (CHH). Comp. Biochem, Physiol., 87: 423-425.

Steele, J.E., 1961. Occurrence of a hyperglycemic factor in the corpus cardiacum of an insect. Nature, 192: 680-681.

Steele, J.E., 1963. The site of action of insect hyperglycemic hormone. Gen. Comp. Endocrinol., 3: 46-52.

Telford, M., 1975. Blood glucose in crayfish. III. The sources of glucose and role of the eyestalk factor in hyperglycemia of *Cambarus robustus*. Comp. Biochem. Physiol., 51: 69-73.

Van Harreveld, A., 1936. A physiological solution for fresh water crustaceans. Proc. Soc. Exp. Biol. Med., 34: 428-432.

Vrba, R. and W. Cannon, 1970. Molecular weights and metabolism of rat brain proteins. Biochem. J., 50: 745-753.

Vrba, R. and A. Winter, 1972. Movement of U-^{14}-C-glucose carbon into and subsequent release from lipids and high molecular weight constituents of rat brain, liver and heart *in vivo*. Can. J. Biochem., 50: 91-105.

Vrba, R. and A. Winter, 1973. Amount of ^{14}C assimilated in different organs and tissues of rat after a single injection of U-^{14}-C-glucose. Can. J. Biochem., 51: 93-100.