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Hyperglycemic effects of biogenic amine in the freshwater giant prawn, *Macrobrachium rosenbergii*

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Hyperglycemic Effects of Biogenic Amines in the Freshwater Giant Prawn, *Macrobrachium rosenbergii*

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中文摘要

X-器官-靜竇腺複合體合成釋放甲殼類促進高血糖激素，主要在於調節醣類代謝。生物胺及神經胜　調節物質調控 CHH 之釋放，淡水長臂大蝦，不論是完整或雙眼柄剪除個體，自水溫攝氏 28 度急速曝露於低溫（攝氏 14 度），2 小時內血淋巴血醣量確有明顯的上升。因此，高血糖可認為是低溫壓迫刺激下之典型反應，亦顯示生物胺參與該壓迫反應。腎上腺素、多巴胺、血動情素之作用經由眼柄中 CHH，但是低溫下高血醣反應並非全部經由 CHH 之途徑，其他因子諸如正腎上腺素及 Octopamine 很可能參與高血醣反應。

關鍵詞：週日韻律、生物胺、低溫壓迫、淡水長臂大蝦

Abstract

Crustacean hyperglycemic hormone (CHH), a neurohormone synthesized and released from the x-organ sinus gland complex, is primarily involved in carbohydrate metabolism, and biogenic amines and peptidergic neuroregulators are known to modulate the release of CHH. Marked elevations of hemolymph glucose titers, which peaked within 2 hr, were observed in both intact and bilaterally eyestalk-ablated prawns, *Macrobrachium rosenbergii*, when they were transferred directly from their optimal temperature of 28 ℃ to lower temperatures close to the lethal limit. Hyperglycemia can therefore be considered a characteristic response in this species under cold shock. Involvement of biogenic amines in the hyperglycemic response was also demonstrated. Hyperglycemic effects of epinephrine, dopamine and serotonin were mediated through CHH at the eyestalk level, but the response under cold shock was not exclusively mediated through CHH. It is suggested that factor(s) other than CHH are involved in the hyperglycemic response, possibly norepinephrine or/and octopamine.

Key Words : Circadian rhythm, biogenic amines, cold shock, freshwater giant prawn

Rationale and Purposes

Neuropeptides synthesized in the x-organ sinus gland complex play important roles in regulating physiological processes in crustaceans. Among them, crustacean hyperglycemic hormone (CHH), first reported as a diabetogenic factor in the eyestalk of decapod crustaceans by Abramowitz et al. (1944), was isolated and

characterized on the basis of its amino acid sequence (Kegel et al. 1989). CHH is primarily involved in carbohydrate metabolism in crustaceans and exerts its action by stimulating glycogenolysis in muscle and the midgut gland, and by inhibiting glycogen synthesis (Sedlmeier 1982; Keller and Sedlmeier 1988; Keller and Orth 1990).

Biogenic amines and peptidergic neuroregulators have been found to modulate the release of various neurohormones from crustacean neuroendocrine tissues (Lüschen et al. 1993). Dopamine (DA) and 5-hydroxytryptamine (serotonin, 5-HT) were effective in inducing hyperglycemic responses in *Orconectes limosus* and *Penaeus monodon* (Keller and Beyer 1968; Kuo et al. 1995), and the effects of 5-HT and dopamine in this regard are mediated through the release of CHH in eyestalks. On the contrary, 5-HT was also found to act hyperglycemically in eyestalk-ablated *Carcinus maenas* (Baúchau and Mengeot 1966). In the same species, eyestalk removal alone led to a considerable hyperglycemia within a few hours which persisted at the same level for two days (Lüschen et al. 1993). The possibility that biogenic amines modulate the hyperglycemic response independent of CHH is therefore postulated. The observation suggested that bilateral eyestalk removal can result in hyperglycemia in the presence of a hypoglycemic factor in the eyestalk ganglion of the crab, *Paratelphusa jaquemontii* (Rangneker et al. 1961). This suggestion still remains to be confirmed.

Dopamine (DA) was identified as a stimulatory modulator of CHH release. Octapamine (OA) and 5-HT were potent elevators of glucose levels in intact and eyestalkless shore crabs (Lüschen et al. 1993). The catecholamines epinephrine and norepinephrine were much weaker stimulators, but are also effective in eyestalk-ablated animals. The possibility of aminergic control of glucose elevation in the hemolymph of eyestalk-ablated individuals in association with neuropeptidergic regulation is consequently postulated (Lüschen et al. 1993). This paper presents data on hyperglycemic responses of *M. rosenbergii* to biogenic amines.

Results and Discussion

Circadian changes in hemolymph glucose

Circadian changes in hemolymph glucose concentrations of *M. rosenbergii* were demonstrated in intact prawns, with hemolymph glucose titers peaking at 1.81±0.23 mmol L^{-1} at dawn (0600 hr) (Fig. 1). A secondary peak in hemolymph glucose titers (1.45±0.16 mmol L^{-1}) was observed at 1500 hr. Hemolymph glucose remained rather constant, in the range of 0.96 and 1.20 mmol L^{-1}, during the remaining period. The rhythmic changes in the hemolymph glucose levels became obscured in the eyestalk-ablated individuals. To mimimize any possible errors associated with the cyclic physiological phenonemon of its own, experiments in this study were all conducted at 1500 hr, at which the hemolymph glucose titers were the highest in the photophase of the 12L/12D photoperiod regime. Rhythmicity of physiological processes under the influences of cyclic changes in photoperiod regime have often been reported. A circadian rthymicity of hemolymph

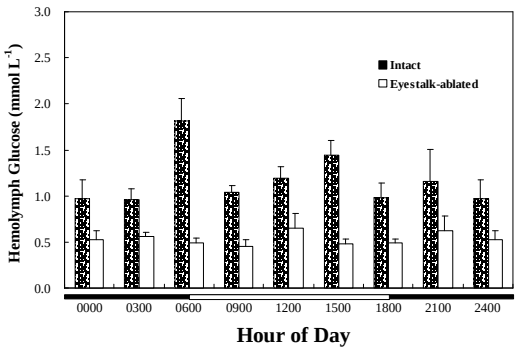

Fig. 1. Circadian changes in hemolymph glucose titers (Mean ± SEM, N=6) of freshwater giant prawns, *Macrobrachium rosenbergii*, under a photoperiod of 12L/12D and a temperature of 28 ℃.

glucose titers in intact, but not in eyestalk-

ablated *M. rosenbergii* was observed in this study. The observations suggest that the rhythmic changes in hemolymph glucose concentration is intimately influenced by rhythmic changes in CHH titers. By use of radioimmunoassay, Santos and Keller (1993) showed that changes in hemolymph glucose in *Orconectes limosus* were preceeded by significant alterations in hemolymph CHH titer. Circadian rthymicity in both CHH and glucose level in the hemolymph were also reported elsewhere (Kallen et al. 1990; Keller and Orth 1990).

Hyperglycemic effects of biogenic amines

Intact prawns which had been acclimated to 28 ℃ for 2 weeks, were directly transferred to 23, 20, 18 and 15 ℃ for 30 min, and the hemolymph glucose titers of each prawn were monitored (Fig. 2). Hemolymph glucose concentration was 1.30±0.21, 2.21±0.13, 2.26±0.38, 2.32±0.41

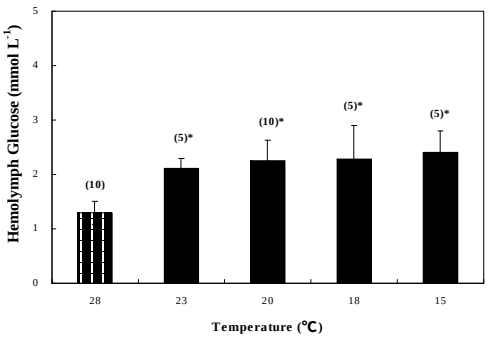

Fig. 2. Hyperglycemic responses of freshwater giant prawns, *Macrobrachium rosenbergii*, under cold shock for 30 min. Number of prawns at each temperature in parenthesis. * mean values are different from the control (28℃) at p<0.01.

and 2.41±0.40 mmol L^{-1} at 28 ℃(control), 23 ℃, 20 ℃, 18 ℃ and 15 ℃, respectively. There was significant elevation of hemolymph glucose of prawns exposed to the temperatures below 23 ℃, relative to controls (28 ℃) (t-test, p<0.01).

The glycemic response of the prawns to various biogenic amines was investigated at 28 ℃. The amines included DOPA, dopamine (DA), norepinephrine (NE), epinephrine(E), serotonin (5-HT), and octopamine (OA). Both intact and eyestalk-ablated prawns were injected with respective amines at the dose of 2 nmol each in 25 μl vehicle (10 mmol L^{-1} phosphate buffer containing sodium mono- and diphosphate, 0.9% NaCl, pH 7.2) at 28 ℃, and the hemolymph glucose concentrations were measured 30 min after administration. Hemolymph glucose elevations caused by injection of phosphate buffer in the intact prawns were noted. An injection of vehicle alone increased the hemolymph glucose titer in intact prawns by up to 50.1 % in 30 min, but only by 0.4 % in eyestalk-ablated individuals. Hemolymph glucose depressions consequent upon eyestalk ablation were also demonstrated, but the glucose elevation by injection of the vehicle was negligible in eyestalk-ablated individuals (Table 1). The hyperglycemic response indicated by the hemolymph glucose elevation was correlated to the dose of sinus gland (SG) extracts administered, and peaked at the injection dose of 0.5 SG equivalent (Lin et al. 1998). All the biogenic amines examined, except DOPA, showed hyperglycemic effects in intact individuals and the hemolymph glucose elevations were significantly different from the sham group (t-test, P<0.05). Among them, dopamine and 5-HT were the most potent in their hyperglycemic effect.

In eyestalk-ablated prawns, mean hemolymph glucose titer was 1.17± 0.19 mmol L^{-1} and 1.08±0.11 mmol L^{-1} for the prawns injected with NE and OA, respectively. Glucose titers in eyestalk-ablated prawns were lower than in intact prawns on the same treatment, but significantly higher than control and sham groups (0.69 mmol L^{-1}).

Glucose elevation resulting from injection stress from mechanical stimulation or from effects of constituents of the vehicle, is most likely mediated through CHH. CHH can respond rapidly to the inejction of glucose and lactate, and the hormone levels

can rise within a minute after exposure to stress (Orth 1989; Keller and Orth 1990). Glucose and lactate are important metabolites involved in the regulation of CHH release, at least in *C. maenas*. This does not however, exclude the possibility that other substances have a similar role. Also, a direct nervous regulation and/or modulation of CHH release through activation or inhibition of specific neuronal pathway may occur in the process of injection (Santos and Keller 1993). Among the biogenic amines administered, E, DA, 5-HT, NE and OA all induced a notabale glucose elevation in the hemolymph of intact individuals, and 5-HT was the most potent, followed by DA. However, an injection of E, DA or 5-HT in the eyestalk-ablated individuals did induce a hyperglycemic response in the range of 111-120 % of the sham control (saline injection), but the difference in the response was not statistically significant (t-test, $p>0.05$). Effects of E, DA and 5-HT on glucose metabolism are primarily mediated through CHH at the eyestalk level. On the contrary, the hyperglycemic responses induced by injection of NE and OA were nearly identical between the intact and eyestalk-ablated individuals. The possibility that the hyperglycemic effects of NE and OA are directly on the target tissue level, but not mediated through CHH is therefore suggested.

Hyperglycemic responses have been most widely documented as a secondary stress response in fish (Pickering and Pottinger 1995). The ubiquitous nature of this response to stress is in not doubt, but no consenus exists regarding the mechanisms involved. Stress-induced hyperglycemia occurs in *Macrobrachium rosenbergii* under cold treatments. It is known that CHH, synthesized and released from the x-organ sinus gland complex in crustaceans, is an important neurohormone involved in glucose metabolism, and biogenic amines have been found to modulate the release of CHH, and other neurohormones such as pigment-concentrating and -dispersing hormones, neurodepressing hormone and vitellogenesis-stimulating hormone**.** The bilateral ablation of eyestalks in *M. rosenbergii* resulted in a decrease in hemolymph glucose level, yet, cold shock markedly elevated hemolymph glucose up to 3.8 and 4.9 times the original level when eyestalk-ablated prawns were exposed to 20 °C and 15 °C for 2 hr. Under the same conditions, the hemolymph glucose of intact prawns was elevated only 2.6 times the control. This suggests that the hyperglycemic response of prawns under cold shock was not exclusively mediated through the actions of CHH at the target tissues, but that other factor(s) are involved, of which NE or / and OA are prime candidates.

Only the parts of research accomplishments, in accordance to proposed scope of research, are presented in in this progress report. The full text of research results will be submitted and published in Journal of Comparative Physiology in the near future.

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Table 1 . Hyperglycemic effects of biogenic amines in intact and eyestalk-ablated freshwater giant prawns, *Macrobrachium rosenbergii.*

	Intact				Eyestalk-ablated			
	Mean ± SEM (mmol L^{-1})	(N)	Percent Response	t-value	Mean ± SEM (mmol L^{-1})	(N)	Percent Response	t-value
Control	1.17 ± 0.09	(37)	66.6		0.69 ± 0.06	(27)	99.6	
Saline	1.75 ± 0.20	(22)	100.0		0.69 ± 0.10	(20)	100.0	
Epinephrine	2.72 ± 0.23	(18)	155.3	3.21 **	0.76 ± 0.12	(16)	110.5	0.47 NS
DOPA	1.96 ± 0.27	(20)	111.8	0.63 NS	0.59 ± 0.08	(14)	85.6	-0.71 NS
Dopamine	2.99 ± 0.30	(11)	170.5	3.53 **	0.83 ± 0.15	(11)	119.8	0.79 NS
Serotonin(5-HT)	3.66 ± 0.30	(5)	208.7	4.36 **	0.77 ± 0.33	(5)	111.3	0.30 NS
Norepinephrine	2.78 ± 0.30	(11)	158.9	2.98 **	1.17 ± 0.19	(5)	169.3	2.15 *
Octapamine	2.62 ± 0.37	(16)	149.5	2.22 *	1.08 ± 0.11	(22)	155.9	2.63 *

Remarks: Saline is represented by phosphate buffer; Injection dose of biogenic amines was 2 nmol per prawn.
* and ** : mean values are different from sham control (saline) at 5% and 1% significance level, respectively (t-test);
NS : not significantly different from sham control.