行政院國家科學委員會專題研究計畫成果報告

淡水長臂大蝦壓迫生理反應之研究

Stress Response of freshwater giant prawn, *Macrobrachium rosenbergii*

計畫編號：NSC-88-2311-B-002-053
執行期限：八十七年八月一日至八十八年七月三十一日

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一、中文摘要

生物胺及神經胜　調節物質調控 x-**器官-靜竇腺** CHH **之合成釋放，完體或雙眼柄剪除淡水長臂大蝦，自水溫** 28 **℃急速曝露於低溫**(23-15**℃**)**,** 2 **小時內血淋巴血醣量確有明顯的上升**, **高血糖可認為是低溫壓迫刺激下之典型反應。亦腎上腺素、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 **也參與高血醣的壓迫反應**, **顯示生物胺參與該壓迫反應。**

關鍵詞：生物胺、低溫壓迫、淡水長臂大蝦

Abstract

Crustacean hyperglycemic hormone (CHH), synthesized and released from the x-organ sinus gland complex, is primarily responsible for carbohydrate metabolism, and biogenic amines and peptidergic neuro-regulators are known to modulate the release of CHH. Hyperglycemia, a characteristic response, were observed in both intact and bilaterally eyestalk-ablated prawns, *Macro-brachium rosenbergii* 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. The fact that factor(s) other than CHH are also involved in the hyperglycemic response.

Keywords: biogenic amine, hyperglycemia, cold shock, giant freshwater prawn

Rationale and Purposes

Custacean hyperglycemic hormone (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).

Hyperglycemia as a secondary stress response, has been documented in most species of fish in response to a wide range of stresses. Elevated blood glucose can result from a reduced utilization of glucose or stimulation of glyconeogenesis and / or glycogenolysis. In crustaceans, elevation of hemolymph glucose are also observed in vivo when they are submitted to stressful conditions, such as exposure to hypoxia or cadmium in red swamp crayfish, *Procam-barus clarkii* (Reddy *et al.* 1994) and in fiddle crab, *Uca pugilator* (Reddy *et al.* 1996) , organic pollutant, naphthalene in *Uca pugilator* (Reddy *et al.* 1996), DDT in freshwater crab, *Barytelphusa guerini* (Fingerman *et al.* 1981), anoxia, or hypoxia, starvation and elevated temperature in Orconectes limosus (Orth 1989; Santos and Keller 1993). Homeostasis of hemolymph glucose is maintained by a counteraction between CHH release and carbohydrate metabolites in the hemolymph. During increases in glycolytic flux, lactate may cause a release of CHH by a positive feedback mechanism. The hormone would then stimulate glycogenolysis, thus increasing glucose availability. The excess glucose released may then suppress CHH release

from the X-organ sinus gland complex by a negative feedback (Santos and Keller 1993). The release of various neurohormones in crustaceans are found to be modulated by Biogenic amines and peptidergic neuro-regulators (Lüschen *et al.* 1993). 5-hydroxy-tryptamine (serotonin, 5-HT) and dopamine (DA) were effective in inducing hyper-glycemic response 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 is mediated through the release of CHH in eyestalks. In contrary, 5-HT was also found to act hyper-glycemically in eyestalk-ablated *Carcinus maenas* (Banchau and Mengeot 1966). In the same species, eyestalk removal alone led to a considerable hyperglycemia within a few hours and remained at the same levels for 2 days (Lüschen *et al.* 1993) The possibility of biogenic amines to modulate hyperglycemic response in a CHH independent manner is therefore postulated. Bilateral eyestalk removal resulted in hyperglycemia and the observation suggested that a hypoglycemic factor was likely present in the eyestalk ganglion of the crabs, *Paratelphusa jaque-montii* (Rangneker *et al.* 1961). The amin-ergic control of hemolymph glucose titer in the shore crab *Carcinus maenus* offers addi-tional possibilities to elevate glucose titer in association with neuropeptidergic control (Lüschen *et al.* 1993). 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 crab. The catechol-amines, epinephrine and norepinephrine were much weaker stimulators, but are also effective in eyestalk ablated animals. The purpose of this research is aimed to elucidate hyperglycemic responses of *M. rosenbergii* under cold shock and to discuss the possible involvements of biogenic amines in the responses.

Results and Discussion

Hyperglycemic responses under cold shock

Hyperglycemic responses of the intact prawn, which had been acclimated at 28 ℃ for 2 weeks and then were directly exposed to cold shock at 23, 20, 18 and 15 ℃ for 30 min, were demonstrated (Fig. 1). The hemolymph glucose concentration was mea-sured 23.38 ± 3.71, 38.17 ±2.38, 40.64 ±6.93, 41.73 ±7.31 and 43.44 ±7.02 mg/dl at 28 ℃ (control), 23 ℃, 20 ℃, 18 ℃ and 15 ℃, respectively. There was significant elevation of hemolymph glucose of the prawns, exposed to the temperatures below 23 ℃, relative to the control (28 ℃) ($p<0.01$). Hemolymph glucose appeared to be highest at 15 ℃, but it was not statistically different from other experimental groups ($P>0.05$).

The hemolymph glucose of intact prawns acclimated under 28 ℃ remained rather contant at the level between 19.84 ±3.54 and 26.36 ±2.05 mg/dl over 6 h period, while there were marked hypergly-cemic responses of the prawns at 20 ℃ and 15 ℃ (Fig. 2). Hemolymph glucose in intact individuals reached the peaks in 2 h, 50.97 ±9.65 mg/dl for 20 ℃ group and 51.50 ±10.26 mg/dl for 18 ℃ group, followed by gradual decreases in the hemolymph glucose at both temperatures. After 6 h of cold shock, hemolymph glucose had dropped to 31.73 ±11.33 mg/dl for 20 ℃ and 32.67 ±11.54 mg/dl for 18 ℃, as compared to 26.07 ±1.55 mg/dl for the control (Fig.2).

The hyperglycemic responses of intact and eyestalk-ablated prawns to cold shock were further monitored for 2 h (Fig. 3). Mean hemolymph glucose of eyestalk-ablated prawns were 13.90 ±1.42 mg/dl, which is lower than those in intact prawns (19.84 ±3.54 mg/dl). The decline in the hemolymph glucose resulted from the eyestalk ablation for 2 h was clearly demonstrated. The hyperglycemic respon-ses of eyestalk-less individuals were more pronounced than those of intact prawns. The hemolymph glucose of eyestalk-less prawns under 20 ℃ increased from 13.90 ±1.43 mg/dl to 53.42 ±6.16 mg/dl in 2 hr

cold shock period. In the same period, the hemolymph glucose increased 4 to 5-folds, from 13.90 ±1.43 mg/dl to 53.42 ±6.16 mg/dl under 20 ℃ and 18 ℃, respectively.

Hyperglycemic response related to crustacean hyperglycemic hormone in eyestalk.

The hemolymph glucose of intact individuals was always higher than those of eyestalk-ablated individuals at three different temperatures at the end of 1 h cold shock, Hemolymph glucose concentrations in both intact and eyestalk-ablated individuals was respectively measured as 23.79 ±3.71 and 13.90 ±1.43 mg/dl at 28 ℃, 41.26 ±8.16 and 36.02 ±3.31 mg/dl at 20 ℃, and 47.79 ±7.36 and 46.83 ±3.87 mg/dl at 15 ℃, all significantly elevated as the temperature dropped from 28 ℃ to 20 ℃ or 15 ℃. The decrease in hemolymph glucose associated with the CHH removal was 40.53 %, 12.70% and 2.0 % at 28 ℃, 20 ℃ and 15 ℃ respectively. A nearly-linear increase in the hemolymph glucose was observed during 2 hr cold shock treatments, i.e., the glucose contents were notably elevated as the time proceeded. The hyperglycemic response of the prawns under 18 ℃ was significantly higher than those under 20 ℃ (Fig. 3). The observed decrease in the hemolymph glucose of eyestalk-ablated prawns was certainly attributed to the removal of CHH, and in the meantime, cold shock alone evidently induced the hyperglycemic response. Observations clearly suggested that factors other than CHH are involved and played a significant role in the hyperglycemic responses in *M. rosenbergii* under cold shock.

Involvement of biogenic amines in hyperglycemic responses

The glycemic response of the prawns to various biogenic amines were further investigated under 28 ℃. The amines included DOPA, dopamine (DA), norepinephrine (NE), epinephrine(E), serotonin (5-HT), and octopamine (OA). Both intect and eyestalk-ablated prawns were injected with respective amines at the dose of 2 nM each in 25 μl vehicle (10 mM phosphate buffer containing sodium mono- and diphosphate, 0.9% NaCl, pH 7.2) at 28 ℃, and the hemolymph glucose contents were quantified 30 min after administration. Hemolymph glucose elevations caused by injection of phosphate buffer in the intact prawns were noted. The elevation was found more eminent when the buffer was made up by potasium mono- and diphosphate (unpublished data). Hemolymph glucose depressions by eyestalk ablation were also demonstrated, but the glucose elevation by injection of the vehicle was negligible in eyestalk-less individuals (Fig. 4). 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 on the intact individuals and the hemolymph glucose elevations were significantly different from the sham group statistically (*t*-test, $P<0.05$). Among them, dopamine and 5-HT were the most potent in hyperglycemic effect.

In eyestalkless prawns, the hemolymph glucose titer was measured at 21.05 ±3.40 mg/dl and 19.39 ±1.91 mg/dl for the prawns injected with NE and OA, respectively, the glucose titers in eyestalk-ablated prawns were much lower than the intact prawns of respective treatments, but significantly higher than control and sham groups (12.39 ±1.10 and 12.43 ±1.81 mg/dl), respectively.

In *M. rosenbergii*, temperatures above 35 ℃ or below 14 ℃ are generally lethal, 18-22 ℃ markedly stunts growth, and 29-31 ℃ is considered optimal (New 1995). Regardless salinity, the temerpature about 13 ℃ is lethal, and a minimum of 18 ℃ is required for growth (Avault 1986). The optimal temperature was reported in the

range of 25-30 ℃ (Brock, 1990) or about 28 ℃ (Sandifer *et al.* 1983) . Hyperglycemic responses of intact prawns were prominent when they were transferred directly from the acclimation temperature of 28 ℃down to 15 ℃, closer to the lower lethal limit (14 ℃) of this species. A similar trend in the hemolymph glucose elevation was observed in 2 hr at 20 ℃ and 18 ℃, followed by a gradual decline in the glucose content thereafter. Changes in the hemolymph glucose reflects the process of physiological compensation to the temperature shock, and the amptitude of glucose flux as a source of energy, is correlated to the temperature preferum of the species concerned, i.e. hyperglycemic response reached the highest level at the lowest limit for the species growth or even to the lethal limit. Ablation of eyestalk under these temperatures resulted in a detectable decline in the hemolymph glucose titers. The hemolymph glucose of eyestalk-less prawns exposed to 28 ℃ was lower than that of intact prawns, and the extent of glucose depression was statistically different ($P<0.05$). However, the increase in the hemolymph glucose of eyestalk-less prawns at 20 ℃ is clearly indicated, and the hemolymph glucose titer of these prawns is higher than that of intact individuals in 2 hr. The elevation of glucose titers of eyestalk-less prawns is much eminent under the cold shock of 18 ℃ and the resultant glucose levels ineyestalk-less group are higher than intact prawns. Evidences suggest that under cold shock, hyperglycemic responses of intact prawns are mediated through CHH, which, under the modulation of biogenic amines, is synthesized and released from x-organ sinus gland complex in the eyestalks. The factors other than CHH , possibly act directly on the carbohydrate metabolism at the tissue level were also implied.

In an attempt to determine involvement of biogenic amines in regulating glucose metabolism directly on the target tissues level or mediated through CHH actions, various biogenic amines at the dose of 2 nmol per prawn were administered in both intact and eyestalk-ablated prawns. An injection of vehicle alone increased the hemolymph glucose titer in intact prawns up to 50.14 % in 30 min, but only 0.37 % in the eyestalk-less individuals. Glucose elevation resulted from the injection stress in the manner of mechanical stimulation or vehicle constituent's effects, is most likely mediated through CHH. CHH can respond reapidly to the inejction of glucose and lactate, and the hormone levels can rise within minute after exposure to stress (Orth 1989; Keller and Orth 1990). Glucose and lactate are found to be important metabolites involved in the regualtion of CHH release, at least in *C. maenas*. This does not however, exclude the possibility that other substance 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 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 is the most potent in the hyperglycemic effects, followed by DA. However, an injection of E, DA, 5-HT in the eyestalk-less individuals did induced hyperglycemic responses in the range of 110.5-119.8 % over the sham control (saline injection), but the difference in the response was statistically insignificant (*t*-test, $p<0.05$). Effects of E, DA, 5-HT on the glucose metabolism are primarily mediated through CHH at the eyestalk level. In contrary, the hyperglycemic responses induced by an injection of NE and OA are nearaly identical between the intact and eyestalk-less 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.

Hyperglyceimic response has been most widely documented as a secondary stress responses in fish. 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 release from X-organ sinus gland complex in crustacean, is an important neurohormone, which is primarily involved in glucose metabolism in the hemolymph, and biogenic amines have been found to modulate the release of CHH and others. The ablation of bilateral eyestalks in *M. rosenbergii* resulted in the decrease in the hemolymph glucose level, yet, cold shock did remarkably elevate the hemolymph glucose up to 3.84 and 4.87 times over the original level when eyestalk-ablated prawns respectively exposed to 20 ℃ and 18 ℃ for 2 hr. Under the same conditions, the hemolymph glucose of intact prawns elevated only up to 2.56-2.60 times over the original. This obviously suggested that the hyperglycemic response of the prawns under the cold shock was not exclusively mediated through the actions of CHH at the target tissues, and further suggest a possibility that other factor(s) are involved in this particular event, and NE or / and OA could be the candidates for the hyperglycemic response of this species under cold shock.

Acknowledgements:

This research was supported by the National Science Council, Republic of China to CMK(grant no. NSC 88-2311-B-002-053)

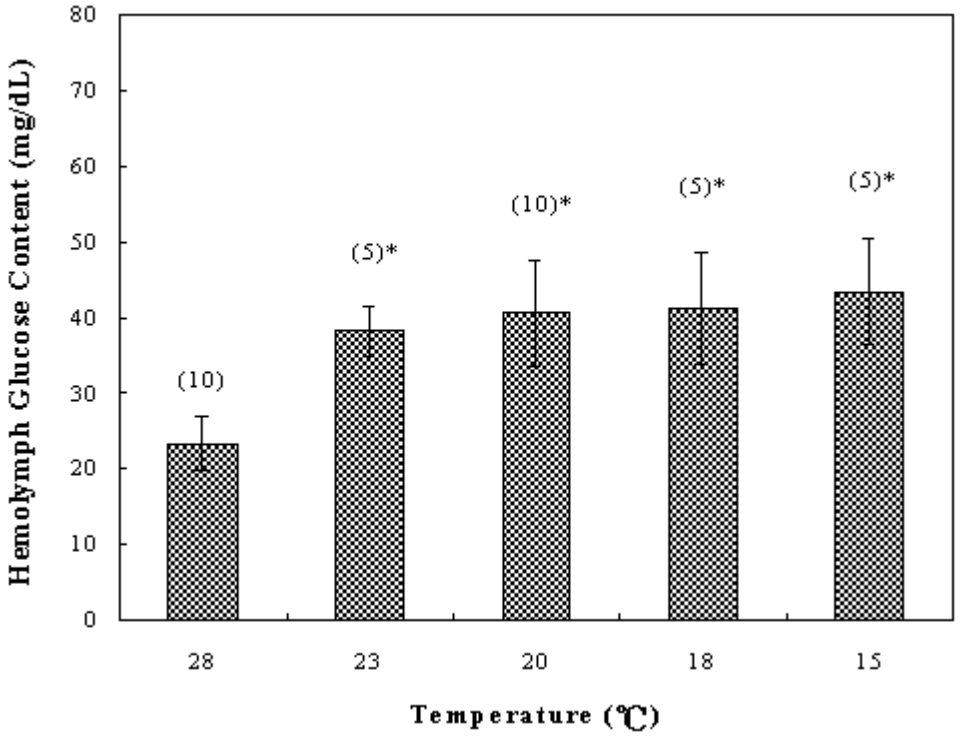

Fig. 1. Hyperglycemic responses of freshwater giant prawns, *Macrobrachium rosenbergii* under cold shock for 30 min. Number of prawns at each temperature in parenthesis. * mean vlaues are different from the control (28 ℃) at P<0.01.

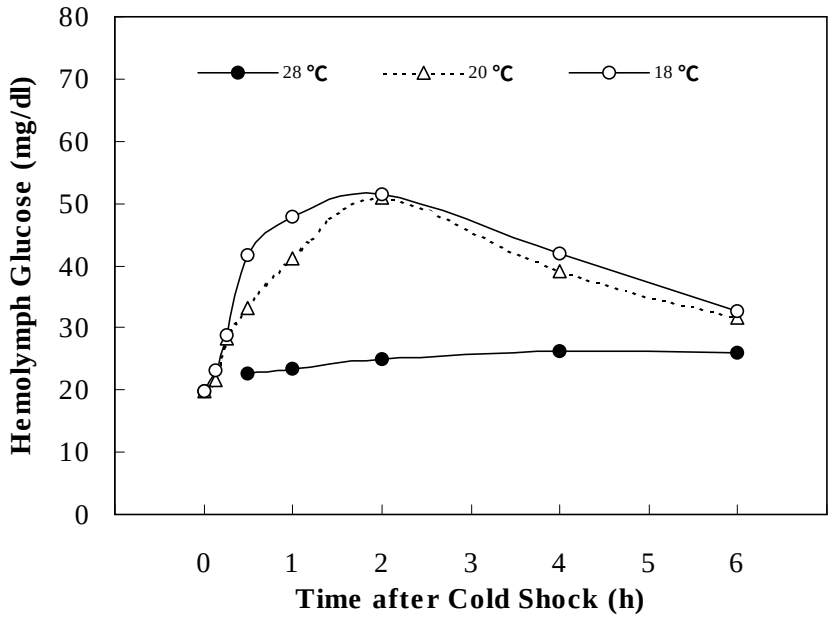

Fig. 2. Time-course changes in hemolymph glucose titers in freshwater giant prawns, *M. rosenbergii*, under cold shock for 6 h.

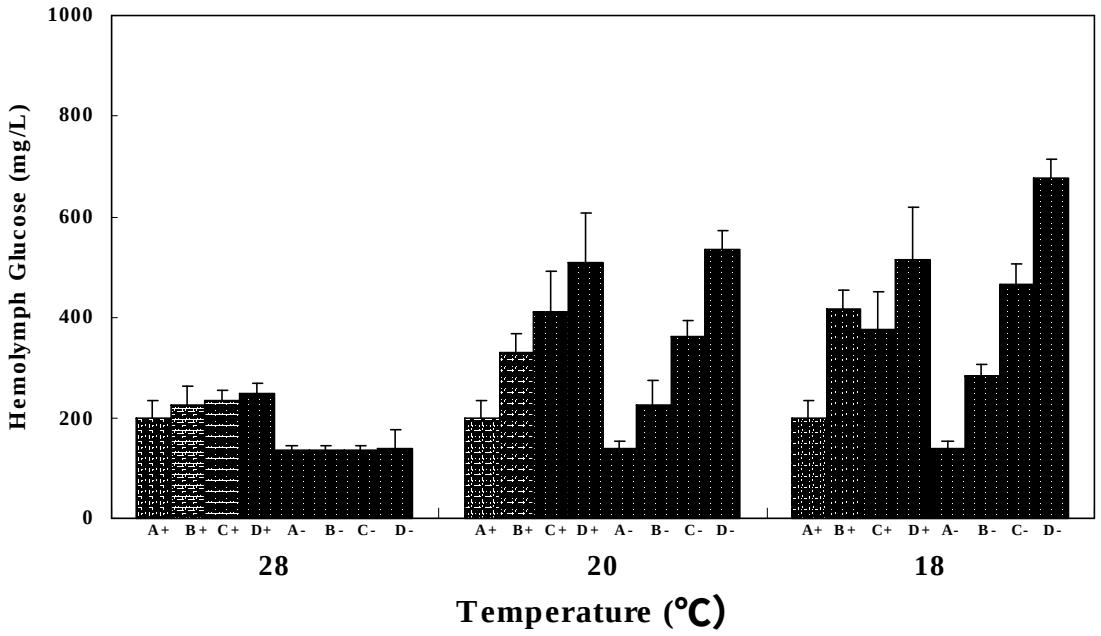

Fig. 3. Hemolymph glucose concentration in intact (+) and eyestalk-ablated (-) freshwater giant prawns. *M. rosenbergii*, when directly transferred from 28 °C (control) to 20 °C or 15 °C for 0 h (A), 0.5 h (B), 1 h (C) and 2 h (D).

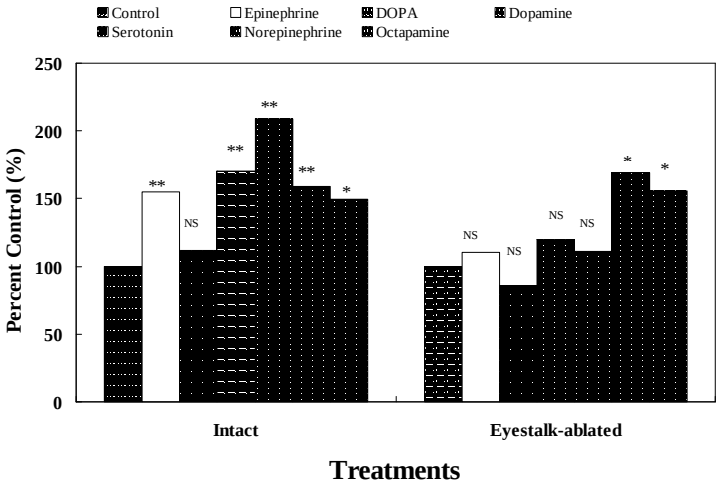

Fig. 4. Hyperglycemic effects of biogenic amines in intact and eyestalk-ablated freshwater giant prawns, Macrobrachium rosenbergii. Control is represented by phosphate buffer. Injection dose of biogenic amines was 2 nmol per prawn (NS not significantly different from sham control, while * and ** represented that the mean values are different from sham control at 5 % and 1 % significant level by t-test, respectively).