Organotin-Induced Hyperglycemia in the Crab, *Oziotelphusa senex senex* Fabricius

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Injection of three different organotin compounds such as tripalmitin, fentin and fenbutatin produced a significant increase in the hemolymph sugar level of intact crabs at *Oziotelphusa senex senex* apparently by stimulating release of the hyperglycemic hormone (HGH).

Organotins and their degradation products which leach out from the painted surfaces occur at detectable levels in sea water, sediments, estuaries and harbours posing threat to non-target species (Hodge *et al.*, 1979; Margaillan *et al.*, 1985; Langston *et al.*, 1987). Even though organotins are found to be neurotoxic to rats (Desaiah and Ho, 1979; Ali *et al.*, 1986), the molecular mechanisms of toxic action are not fully understood. These compounds have been shown to inhibit oxidative phosphorylation (Aldridge *et al.*, 1977), depress thymus dependent immunity (Seinen *et al.*, 1979), and inhibit basal as well as calmodulin activated Ca^{2+} ATPase in rat synaptosomes (Rao *et al.*, 1987).

In decapod Crustaceans, hemolymph sugar level is regulated by a hyperglycemic hormone (HGH). Abramowitz *et al.* (1944) were the first to determine the presence of hemolymph sugar regulating principle in *Callinectes* and called as diabetogenic factor. Since then, a good amount of work has been carried out on the chemical nature, mode and site of action of the HGH (Keller *et al.,* 1985; Sedlmeier, 1985; Liu *et al.,* 1998; Sithigorngul *et al.,* 1999). The amino acid composition of HGH from several crustaceans has been reported (Kleinholz, 1975; Keller and Wunderer, 1978; Keller, 1981; Newcomb, 1983; Huberman and Aguilar, 1986; Soyez *et al.,* 1990; Yasuda *et al.*, 1994; Ohira *et al.,* 1997; Marco *et al.,* 1998). Recently, the gene for HGH was also cloned from several crustaceans (Reddy and Ramamurthi, 1999). We have determined the expression of HGH gene at different molt and reproductive stages (Reddy *et al.,* 1997). HGH, produced by neurosecretary cells belonging to the X-organ, stored in and released from the sinus gland located in the eyestalks, is the major hormonal regulator of carbohydrate metabolism in crustacea.

Although there has been considerable work on HGH in a number of species, data are limited for the factors regulating the release of HGH. The release of HGH is influenced by external factors such as photoperiod, temperature, nutrition and stress (Kleinholz and Keller, 1979; Van Herp and Payen, 1991) as well as internal factors (Fingerman, 1995; 1997). We have demonstrated earlier that xenobiotics such as sumithion and hexachlorocyclohexane induces hyperglycemia in the crab, *Oziotelphusa* and hypothesised that these compounds produce this effect indirectly by stimulating release of HGH. The objectives of the present study are (a) to test our hypothesis generated earlier that xenobiotics induce hyperglycemia in decapod crustaceans by using organotin compounds and (b) to determine for the first time what effect, if any, organotin compounds have on the hemolymph sugar level in *Oziotelphusa, in vivo.*

Adult, inter molt (stage C_4) crabs of *Oziotelphusa senex senex* were collected from paddy fields and irrigation canals (Tirupati). They were acclimatized to the laboratory conditions (temperature 27±1 °C ; RH 75% and a light period of 12 hours) for one week before being used for experimentation. Only male crabs weighing 30–32 g were used in the present study. They were fed daily once *ad libitum* sheep meat and medium was changed 2 h after feeding. Feeding was stopped 24 h before commencing the experiment to overcome differences, if any, due to differential feeding.

Crabs were divided into 10 batches of 9 animals each. The first batch served as control and received no treatment. The second batch of crabs received 10 µl of 10% ethanol and served as controls. The 3rd, 4th and 5th batches were injected with 10 µmoles of tripalmitin, fentin and fenbutatin, respectively, through the arthrodial mem-

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brane of the coxa of 3rd pair of walking legs in 10 µl volume. The organotin compounds obtained from Environmental Protection Agency, Research Triangle Park, USA through a generous gift by Dr. K. S. P.Rao were dissolved in small amount of ethanol and diluted in crustacean saline.

The eyestalks were removed (ESX) by cutting off the organs at the base without prior ligation but with cautery of wound after operation from the remaining batches. Crabs in batch 6 were not given any treatment and used after 24 h post-ablation. Crabs in batch 7 were injected with 10 µl 10% ethanol and served as ESX control. The crabs in batches 8–10 were injected with 10 µmoles of tripalmitin, fentin and fenbutatin, respectively, in 10 µl volume, after 24 h of ESX. The hemolymph samples were collected 2 h after the injection at the same time of the day to avoid influence of circadian rhythm. The hemolymph sugar level was determined following the method of Carroll *et al.* (1956) using anthrone reagent. The data were analysed by using ANOVA and Student Newman-Keul (SNK) test (Steel and Torrie, 1960).

Table I. Hemolymph sugar level in the crab after different treatments.

	Hemolymph sugar level (mg/100 ml)
Intact crabs	33.29 ± 1.24
Ethanol injected intact crabs	32.63 ± 3.88 (– 1.98)
Tripalmitin injected intact crabs	53.83 ± 2.49[a] (61.71)
Fentin injected intact crabs	51.16 ± 2.14[a] (53.68)
Fenbutatin injected intact crabs	39.40 ± 4.16[a] (18.35)
Eyestalkless crabs (ESX)	22.15 ± 1.83[a] (– 33.46)
Ethanol injected ESX crabs	21.11 ± 1.94[a,b] (– 4.70)
Tripalmitin injected ESX crabs	21.07 ± 1.36[a,b] (– 4.87)
Fentin injected ESX crabs	20.83 ± 2.17[a,b] (– 5.95)
Fenbutatin injected ESX crabs	21.21 ± 2.56[a,b] (– 4.24)
F ratio	227.37
p value	< 0.0001

Values are Mean ± S. D. of 9 individual observations. Values in paranthesis are percent change from intact crabs. For calculation of % change for intact-injected and ESX crabs, intact crabs served as controls; For ESX-injected crabs, ESX crabs served as control.
[a] Values are significant at $p<0.0001$ from intact crabs.
[b] Values are not significant from ESX crabs.

Bilateral ESX significantly ($P<0.0001$) decreased (–33.46%) hemolymph sugar level (Table I). The hemolymph sugar levels increased significantly ($p<0.0001$) after injection of organotin compounds into intact animals. Among the three organotins, tripalmitin produced most elevation (61.71%) in hemolymph sugar level. On the contrary, injection of organotin compounds into ESX crabs did not affect the hemolymph sugar level.

Organotin compounds might have induced hyperglycemia in the intact crabs in several different ways such as (a) by triggering release of HGH, (b) by mimicking the action of this hormone or (c) even by directly stimulating glycogenolysis by activating the enzyme phosphorylase. However, given the fact that organotin compounds were not able to produce hyperglycemia in ESX crabs, we favour the hypothesis that organotins exerted its effect by stimulating release of HGH from the sinus glands of eyestalks. This also supports the hypothesis, that the sinus glands in the eyestalks of *Oziotelphusa* are the main release site for HGH.

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