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Influence of Estradiol and 17α-hydroxyprogesterone on Carbohydrate Metabolism of Fresh Water Field Crab *Oziotelphusa senex senex (Fabricius)*

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Abstract: ***Crustacean reproductive physiology is governed by the variety of hormonal and neuronal factors. The present study have been investigated the influence of estradiol and 17α- hydroxyprogesterone on carbohydrate metabolism of the fresh water paddy field crab Oziotelphusa senex senex. The crabs were divided into two experimental groups and injected with estradiol and 17 α-hydroxyprogesterone @ 10 mol/ crabs separately on different intervals of the one month experimental duration. The total carbohydrates, glycogen and hemolymph sugar levels were observed in the hepatopancreas, hemolymph and muscle in different time intervals (7th, 14th and 21st day) in control and experimental groups. In the two different steroids injected crabs, in all most all the cases estradiol injected animals showed highly significant (P<0.01) increase in total carbohydrates, glycogen and heamolymph sugar levels, followed by 17α-hydroxyprogesterone. The possible impact of steroids on carbohydrate metabolism of crustaceans is discussed.***

Keywords: Estradiol, 17 α-hydroxyprogesterone , Carbohydrate metabolism, *Oziotelphusa senex senex*

1. Introduction

Steroids hormones have been reported to be present in the hepatopancreas, ovary, and hemolymph of the crustaceans, their levels changing in correlation with the oocyte maturation cycle (Lafont and Mathieu, 2007). Indeed, a positive correlation between vitellogenin circulating levels and hemolymph levels of progesterone and 17β-estradiol has been reported for crabs (Shih, 1997; Warrier *et al*., 2001; Zapata *et al*., 2003) and shrimps (Quinitio *et al.,* 1994; Yano, 2000). Moreover, the stimulatory effects of some vertebrate-type steroids such as 17β-estradiol and progesterone on ovarian growth in decapods have been reported by several authors. In the crayfish *Macrobrachium rosenbergii*, 17β-estradiol behaved as a metabolic activator at the cellular level, causing an increase in mitochondrial ATP-ase, cytosolic malate dehydrogenase, and glucose-6-phosphate dehydrogenase in the hepatopancreas (Ghosh and Ray, 1993a). In *Procambarus clarkii*, 17β-estradiol and 17α-hydroxyprogesterone produced a significant increase in the gonado somatic index, while only the latter brought about a significant increase in oocyte diameter (Rodriguez *et al*., 2002b). On the other hand, 17α-hydroxyprogesterone, when administered in combination with methyl farnesoate, inhibited oocyte growth by suppressing the stimulatory action of the methyl farnesoate on the ovary of *Procambarus clarkii* (Rodriguez *et al*., 2002a).

Very few reports are available on the effect of vertebrate steroid hormones on the carbohydrate metabolism of crustaceans. Thus the present work has been designed to study the effects of two different steroid hormones, estradiol and 17α-hydroxyprogesterone on total carbohydrates and glycogen levels of hepatopancreas and muscle tissues and hemolymph glucose levels of fresh water crab *Oziotelphusa senex senex.*

2. Materials and Methods

The fresh water field crab, *O.senex senex* (Fabricius) were collected from rice fields of Mahammadapuram village, Nellore district, Andhra Pradesh and maintained in the laboratory at 27 ± 1^{0}C in plastic troughs partially filled with water. They were acclimatized to laboratory conditions for at least 3 days before being used for experimentation. The animals were fed *adlibitum* once every two days with sheep meat and the water in the troughs was replaced daily. Feeding was stopped one day before the commencement of experiments to avoid changes due to prandial activity. Only intact uninjured crabs weighed about 28 – 32 g were used in the present study.

The stock solutions of the estradiol and 17α-hydroxyprogesterone were prepared by dissolving 1 mg of the respective hormone in 1 ml of pure ethanol. The crabs selected were divided into 3 groups of 10 each. The first group received 100 µl injection of crustacean saline which served as control. The second group was injected with estradiol and the third group was injected with 17α-hydroxyprogesteron. The dosage of 100 µl steroids, estradiol, 17α-hyddroxyprogesterone was injected in the second chelate leg one time in a week for four week duration. The hemolymph was collected through the arthrodial membrane of the coxa of the third pair of walking legs by using hypodermic syringe.

The crabs were dissected and the hepatopancreas was quickly isolated. The muscle tissue was isolated from chelate legs. The tissues homogenates were prepared separately for estimation of levels of total carbohydrate, glycogen and determination of hemolymph glucose levels. Total carbohydrate and Glycogen content was estimated by the method of Carrol *et al*., (1956). Hemolymph glucose content was determined by the method of Kemp and Mayers (1954).

3. Results and Discussion

Mean values of total carbohydrate levels obtained from the hepatopancres, hemolymph and muscle tissues of *Oziotelphusa senex senex* from control and experimental groups injected with estrodiol and 17α-hydroxyprogesterone are presented in table 1. The corresponding percent changes in figures 1 to 3. It is clear from the results that the total carbohydrate levels was significantly higher (P<0.01) in haptopanceras, hemolymph and muscle tissues of *Oziotelphusa senex senex* injected with estradiol and 17α-hydroxyprogesterone than compared with controls at different time intervals of experimental duration. Further it is also clear that the magnitude of increase in total carbohydrate level was slightly higher in *Oziotelphusa senex senex* injected with estradiol. Amongst the two steroid hormones injected crabs, estrodiol injected animals showed a grater percent increment in total carbohydrates compared to 17α-hydroxyprogesterone injected crabs (figures. 1, 2 and 3).

Mean values of glycogen content of hepatopancreas and muscle tissues of *Oziotelphusa senex senex* from control and experimental group animals at different time intervals are presented in table 2 and the corresponding percent changes in figures 4 and 5 respectively. It is evident from the results that the glycogen content was significantly higher (P<0.01) in the hepatopancreas and muscle of estradiol and 17α-hydroxyprogesterone injected animals. Although the glycogen content levels in hepatopancreas as well as muscle increased significantly with increase in duration in both control and experimental steroids injected animals. The magnitude of increase was more pronounced in experimental groups than in control group. Maximum percent increase was observed in 21st day of post injection of estradiol injected group followed by 17α-hydroxyprogesterone injected animals (figures: 4 and 5).

Mean values of hemolymph sugar levels recorded at different time intervals of controls and post injection of estradiol and 17α-hydroxyprogesterone separately are presented in table 3, and corresponding percent changes in figure 6. It is clear from the results that the hemolymph sugar levels was significantly higher (P< 0.01) in *Oziotelphusa senex senex* after 6 hours of post injection of both estradiol and 17α-hydroxyprogesterone than from controls. The results also showed that the hamolymph sugar levels increased gradually but significantly (P<0.01) with increase in time duration upto 6 hours of post injection of both estrodiol and 17α-hydroxyprogesterone. However the magnitude of increase was more effective in estradiol injected animals than 17α-hydroxyprogesterone injected group. The maximum percent increment showed in after post injection of 6th hour in estradiol injected group.

The regulation of reproduction in crustaceans is highly diverse and most species maintain separate sexes (Chang and Sagi, 2008; Parnes *et al.,* 2008). The reproductive biology of crustaceans is crucial for the crustacean industry. Reproductive physiology in crustaceans is highly controlled and regulated by the nervous and endocrine systems (Engelmann, 1994). Endocrine control of female reproduction is governed by a variety of hormonal and neuronal factors that involve neuropeptide hormones, such as gonad-stimulating hormone (GSH) and vitellogenesis-inhibiting hormone (VIH) ; terpenoids, such as methyl farnesoate, a stimulator of vitellogenesis; ketosteroids, such as ecdysteroids; and finally sex steroids such as estradiol and progesterone (Huberman, 2000; Zapata *et al*., 2003). Ecdysteroids are primary hormonal factors of molting and positively affect vitellogenesis also (Subramoniam, 2000).

The results obtained clearly suggest that there were significant (P<0.05) increase in total carbohydrates level in hepatopancreas (table 1 and figure 1), hemolymph (table 1 and figure 2) and muscle (table 1 and figure 3) of female field crab *Oziotelphusa senex senex* injected with estradiol and 17α-hydroxyprogesterone at different time intervals suggesting that the steroid hormones plays a positive role in enhancing total carbohydrates of different tissues of *Oziotelphusa senex senex.* Similar results have been reported by Ramachandra Reddy *et al*., (2006), Chang *et al.,* (1993), Reddy and Ramamurthi, (1999) and in different crustaceans. Similar results have also been obtained in other vertebrates and invertebrates Tsukimura (2001) and Wilder *et al.,* (2002). It is possible that steroid hormones have improved carbohydrate metabolism and it may be stimulate the hepatopancreas to produce vitellogenin (Ramachandra Reddy *et al.,* 2006), this is major precursor to egg proteins and carbohydrates. These are than secreted from the hepatopancreas and transported through the hemolymph to ovary.

The results obtained also suggest that there was a steroid specific effect as revealed by percent changes (figures 1, 2 and 3). In the two separate steroid injected animals, in all most all the cases estradiol injected animals showed highly significant (P<0.01) increase followed by 17α-hydroxyprogesterone. It is also probable that estradiol enters cells freely and interacts with a cytoplasmic target cell receptor.

Glycogen is a storage carbohydrate present in tissues, which is useful for production of metabolic energy, (Stetten and Stetten, 1960). In the present study steroid hormones of estradiol and 17α-hydroxyprogesterone elevated total carbohydrates and glycogen levels (table 1.2; figures 1.4 and 1.5) in hepatopancreas and muscle of *Oziotelphusa senex senex*. The glycogen levels in hepatopancreas and muscle of *Oziotelphusa senex senex* increased significantly (P<0.05) after injection of estradiol and 17α-hydroxyprogesterone (table 1.2) . It is evident that the steroid hormones are influenceing the glucogenesis in crustaceans. Similar results have also been reported in *Penaeus indicus* and *Metapenaeus monocerus* injected with methionine-enkephalin (Kishori *et al.,* 2001), *Scylla serrata* (Reddy and Kishori, 2001).

Further in this study injection of estradiol and 17α-hydroxyprogesterone resulted in increase in the hemolymph sugar levels at different time intervals (figure 1.6; table 1.3) of *Oziotelphusa senex senex*. This two steroids elicited hyperglycemic response in *Oziotelphusa senex senex* (table, 1.3). The time course action of these steroids is presented in figure 1.6. Similar results has also demonstrated in the mud crab, *Scylla serrata* (Reddy and Kishori, 2001) injected with

methionine-enkephalin, in fresh water crab *Oziotelphusa senex senex* (Reddy, 1999) and in prawns, *Penaeus indicus* and *Metapenaeus monocerus* (Kishori *et al*., 2001) and in fiddler crab *Uca lacteal annulipes* (Nagaraju and Reddy, 2002) . It may be caused, accumulation of sugar molecules in the tissues and these glucose molecules are ultimately mobilized to hemolymph, causing hyperglycemia. In crustaceans, glucose is the major circulating carbohydrate (Telford 1968: Dall, 1975) and glucoseserves as a precursor for the synthesis of oligosaccharides and glycogen (Meenakshi and Scheer, 1961). Several workers reported that hemolymph glucose levels are influenced by various physiological conditions such as molt cycle (Telford, 1968) and reproductive cycle (Dean and Vernberg, 1965). A rise in hemolymph glucose level is a classical response exhibited by crustaceans to physiological stresses (Dall, 1975).

4. Conclusion

The results of the present study provide the evidence for the involvement of steroid hormones in the regulation of carbohydrate metabolism in the crab, *Oziotelphusa senex senex*. The results also suggest that this hormones induces hyperglycemia by triggering release of hyperglycemic hormone (HGH) from the sinus gland of eyestalks. The released hyperglycemic hormone stimulates the phosphorylase system. The resultant glucose molecules leak from tissues to hemolymph ultimately resulting in hyperglycemia.

Table 1: Mean (±SD; n = 6) values of total Carbohydrates in the hepatopancres, hemolymph and muscle in control and estradiol and 17α-hydroxyprogesterone injected individuals of female field crab *Oziotelphusa senex senex (mg of glucose/ g.wet wt) (mg of glucose/100ml).*

Tissue	*Treatment Duration*	Control	Estradiol injected	17 α – Hydroxy progesterone injected
Hepatopancreas (mg/g. wet.wt)	7th Day	31. 43 ± 1.89	42. 56 ± 2.10	38.12± 2.12
	14th Day	29.43± 2.56	49.21± 1.31	43.12± 1.33
	21st Day	27.19± 2.98	58.42± 2.14	53.33± 1.12
Hemolymph (mg / 100ml)	7th Day	69. 08 ± 1.86	92.21± 1.13	78.12± 2.11
	14th Day	72.11 ± 1.91	107.32 ± 1.19	98.34 ± 1.97
	21st Day	70.93 ± 1.11	122.49 ± 1.87	112.68± 2.32
Muscle (mg/g. wet.wt)	7th Day	6.82 ± 0.56	8.95± 0.63	8.11± 0.32
	14th Day	7.13± 0.61	10.21± 0.31	9.78± 0.63
	21st Day	7. 58± 0.49	12.32± 1.02	10.93± 0.54

Table 2: Mean (±SD; n = 6) values of glycogen in the hepatopancres, and muscle in control and estradiol and 17α-hydroxyprogesterone injected individuals of female field crab *Oziotelphusa senex senex (mg of glucose/ g.wet wt) (mg of glucose/100ml).*

Tissue	*Treatment Duration*	*Control*	*Estradiol injected*	*17 α – Hydroxy progesterone injected*
Hepatopancreas (mg/g. wet.wt)	7th Day	4.59 ± 0.58	6.13± 0.91	5.91± 0.24
	14th Day	4.93 ± 0.31	6.98 ± 0.23	6.30 ± 0.11
	21st Day	5.12 ± 0.80	8.21 ± 0.56	7.42 ± 0.36
Muscle (mg/g. wet.wt)	7th Day	1.68 ± 0.09	1.96 ± 0.06	1.91 ± 0.04
	14th Day	1.73 ± 0.05	1.83 ± 0.09	1.78 ± 0.06
	21st Day	1.72 ± 0.06	2.06 ± 0.04	1.94 ± 0.08

Table 3: Effect of injection of estradiol and 17α-hydroxyprogesterone on the heamolymph sugar levels at different time intervals of post injection (mg glucose/100ml)

Time after injection	*Control*	*estradiol injected*	*17α-hydroxyprogesterone injected*
1 hr	6.97 ± 0.56	11.93 ± 1.24	10.62 ± 1.35
2 hr	7.36 ± 0.69	13.62 ± 1.92	13.11 ± 2.35
6 hr	7.95 ± 0.51	19.21 ± 2.13	18.62 ± 1.32
8 hr	7.23 ± 0.84	14.36 ± 1.65	12.97 ± 2.11

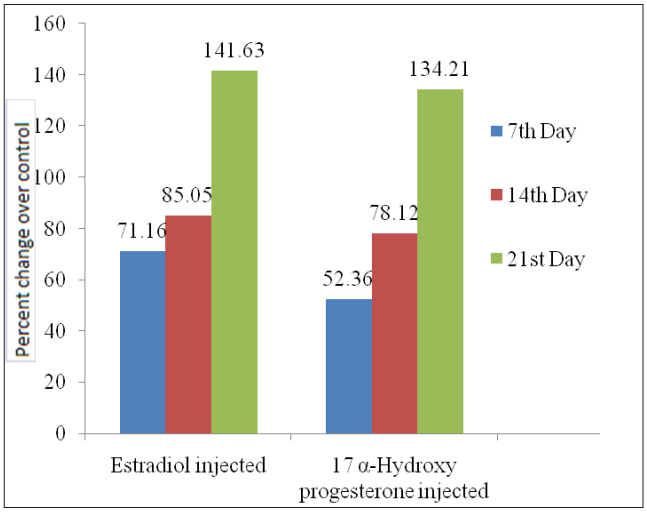

Figure 1: Percent change in total carbohydrate content in the hepatopancreas in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex.*

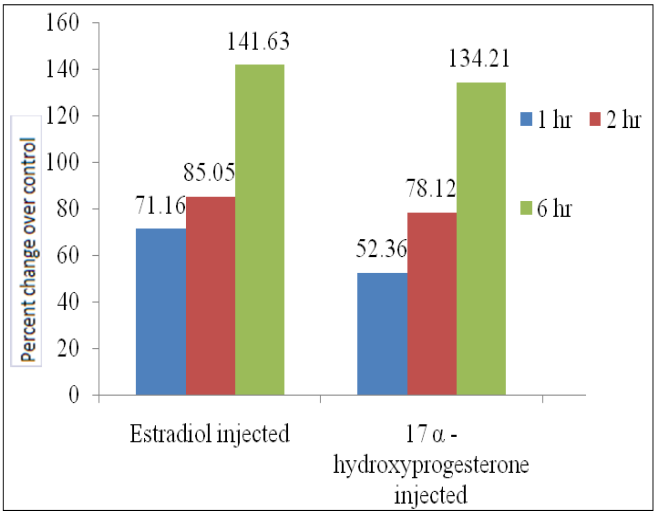

Figure 2: Percent change in total carbohydrate content in the hemolymph in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex.*

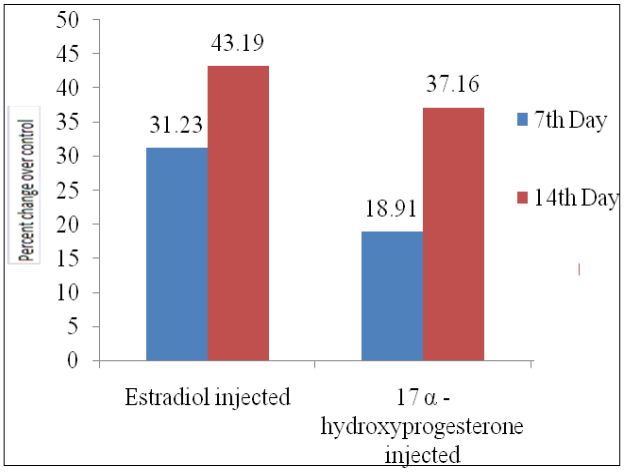

Figure 3: Percent change in total carbohydrate content in the muscle in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex.*

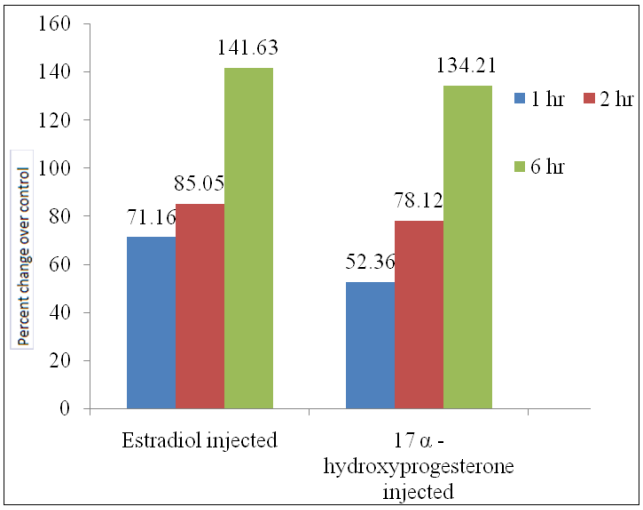

Figure 4: Percent change in glycogen content in the hepatopancreas in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex*

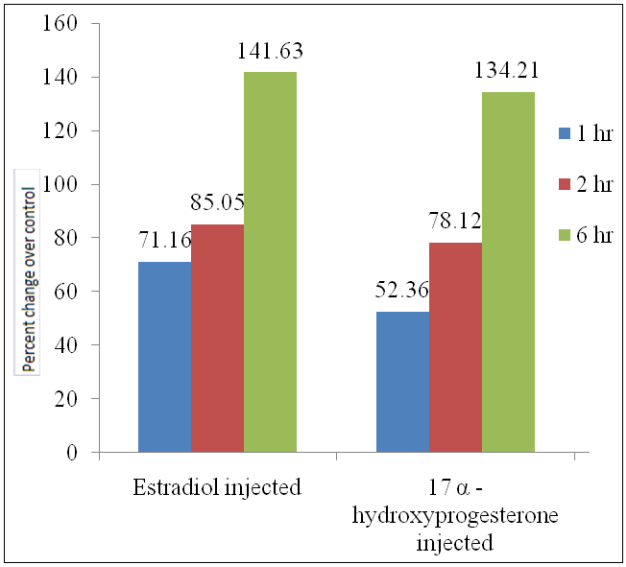

Figure 5: Percent change in glycogen content in the muscle in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex.*

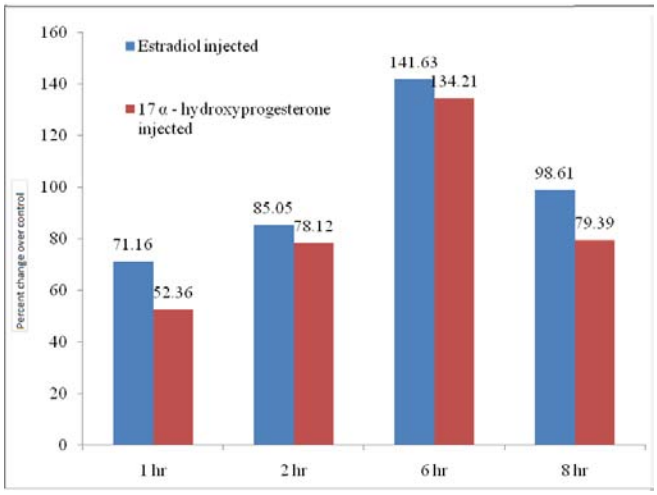

Figure 6: Percent change in hemolymph sugars in response to estrodiol and 17α-hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex.*

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