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CHANGES IN ATPASE AND PHOSPHATASES IN DIFFERENT TISSUES OF CRAB, *OZIOTELPHUSA SENEX SENEX*, FOLLOWING EXPOSURE TO FENVALERATE

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ABSTRACT
The changes in Na^+-K^+-ATPase, $Mg2^+$-ATPase and Acid phosphatase and Alkaline Phosphatase in the Central Nervous System (CNS) and Pedipalpal Muscle (PM) of Crab, *Oziotelphusa senex senex* following ambient exposure to sublethal concentration of Fenvalerate have been studied. The changes in ATPase and Phosphatase systems at different time intervals like 3, 6, 12, 24 and 48h following exposure have been studied. We found that ATPase activity was inhibited in both CNS and PM with maximum effect at 12h after exposure. In contrast, phosphatase system was elevated at all time periods with greater changes at 24h after exposure to Fenvalerate. Thereafter, changes in ATPase and phosphatase system showed recovery tendency by 48h, suggesting operation of detoxification mechanisms on one hand and possible biodegradation of fenvalerate in the animal system.

INTRODUCTION

Pyrethroids are a class of neurotoxic pesticides used widely in agricultural and household practices. Due to their extensive usage, these insecticides are more likely to be toxic to fish and other aquatic non-target organisms (Koprucu and Aydin, 2004; Rahmi Aydin *et al.,* 2005). Some studies have demonstrated the presence of pyrethroid residues in sediments of residential streams (Weston *et al.,* 2011). Moreover occupational exposure of humans to pyrethroids has also been reported (Bradberry *et al.,* 2005). Thus indiscriminate and injudicious use of insecticides has resulted in development of resistance among target species on one hand and deleterious effects on scores of non target animals (Hill, 1985). In this context, the effects of fenvalerate, a widely used agricultural insecticide have been studied on rice field crab, *Oziotelphusa senex senex*, to understand how this could affect the energy metabolism in non-target species.

MATERIALS AND METHODS

Adult male crabs, *Oziotelphusa senex senex,* weighing 30 ± 2g were collected from local paddy fields and freshwater ponds and were acclimatized to laboratory conditions for 2 weeks prior to experimentation. They were fed with pieces of meat *ad libitum* during this period.

Technical grade Fenvalerate, obtained from Rallis (India) Ltd, India was used as the test chemical. The crabs were divided into batches of 10 and were exposed to the ambient medium with different concentrations of Fenvalerate dissolved in acetone for 48h. Mortality at each concentration was recorded and lethal concentration 50 (LC_{50}) was derived by Probit Analysis (Finney, 1971). The LC_{50} calculated by this method was found to be 2.4 ppm and a quarter of this (0.6 ppm) was chosen as a sub-lethal concentration for the present study. After stipulated time, CNS and Pedipalpal muscle were isolated and used for assay of both ATPases and phosphatases.

The specific activity of ATPases was estimated by the method of Fritz and Hamrick (1966) as reported by Desaiah and Ho (1979) with slight modifications and the inorganic phosphates liberated was estimated by the method of Lowry and Lopez (1946) as modified by Phillips and Hayes (1977).

Alkaline phosphatase was estimated by the method of Bodansky (1932). For acidic phosphatase, the procedure followed was same as that of alkaline phosphatase except the substrate (Sodium b-glycerol phosphate) pH was adjusted to 5.0 with dilute acetic acid. The results were also analyzed for statistical significance (Pillai and Sinha, 1978).

RESULTS AND DISCUSSION

The results clearly showed that Na^+- K^+-ATPase and Mg^{2+}-ATPase activity was inhibited in the CNS and PM of crab following ambient exposure to sub-lethal concentrations of fenvalerate (Table 1). Further, the changes in ATPase activities were gradually increased from 3h to 12h with maximum

Table 1: Changes in ATPases (Na^+-K^+ and Mg^{2+}) and Phosphatase (acid and alkaline) activity in the central nervous system (CNS) and pedipalpal muscle (PM) of crab, *Oziotelphusa senex senex* at different time intervals following ambient exposure to sub-lethal concentration of Fenvalerate

S. No.	Parameter	Tissue	Control	Time elapsed after exposure 3 h	6 h	12 h	24 h	48 h
1	Na^+-K^+-ATPase (μmoles of Pi/mg protein) h	CNS	60.81 ± 3.21	57.21 ± 2.62 (-5.94)	54.60 ± 3.01 (-10.31)	46.06 ± 2.81 (-23.81)	55.73 ± 3.76 (-7.44)	58.82* ± 2.79 (-2.31)
		PM	49.26 ± 2.62	46.26 ± 2.71 (-6.09)	44.82 ± 3.01 (-9.01)	35.87 ± 2.02 (-27.18)	42.33 ± 1.98 (14.07)	48.04* ± 2.43 (-2.48)
2	Mg^{2+} ATPase (μmoles of Pi/mg protein) h	CNS	36.80 ± 2.02	34.06 ± 1.93 (-7.45)	29.86 ± 1.82 (-18.86)	27.88 ± 1.69 (-15.63)	31.05 ± 1.58 (-15.63)	34.60 ± 2.18 (-5.98)
		PM	38.46 ± 1.92	35.07 ± 2.02 (-8.81)	32.62 ± 1.78 (-15.18)	30.08 ± 1.86 (-21.79)	33.09 ± 1.72 (-13.86)	37.61* ± 1.88 (-2.21)
3	Acid Phosphatase (μmoles of Pi/mg protein) h	CNS	2.94 ± 0.12	3.10* ± 0.19 (+5.44)	3.24 ± 0.16 (+10.20)	3.32 ± 0.13 (+12.92)	3.64 ± 0.14 (+23.81)	3.08* ± 0.11 (+4.76)
		PM	3.69 ± 0.13	3.79 ± 0.14 (+2.71)	3.92 ± 0.11 (+6.23)	4.18 ± 0.14 (+13.28)	4.56 ± 0.16 (+23.57)	3.84* ± 0.13 (+4.07)
4	Akaline Phosphatase (μmoles of Pi/mg protein) h	CNS	3.98 ± 0.14	4.14* ± 0.12 (+4.02)	4.22 ± 0.11 (+6.03)	4.39 ± 0.12 (+10.30)	4.98 ± 0.13 (+25.13)	4.06* ± 0.12 (+2.01)
		PM	4.12 ± 0.16	4.26* ± 0.15 (+3.39)	4.42 ± 0.12 (+7.29)	4.63 ± 0.14 (+12.18)	5.12 ± 0.18 (+24.27)	4.18* ± 0.12 (+1.46)

Each value is mean ± SD of six individual observations. For each value tissue from six animals was pooled.; Values in parenthesis denote percent change from control; Values are significant at $p < 0.05$, * Not significant.

inhibition at 12h following exposure. Thereafter, the changes showed a recovery tendency through 24h, with near normalcy by 48h in both tissues.

Contrary to ATPase activity acid phospahatase and alkaline phosphatase recorded an elevation in CNS and PM of crab following exposure to fenvalerate (Table 1). Phosphatase activity also showed gradual elevation through 3h, 6h, and 12h, with maximum effect at 24h. These changes after 24h showed a tendency towards normalcy. From the results, it is obvious that the time of maximum change was different between ATPase (12h) and phosphatase (24h) systems.

Mg^{2+}-ATPase catalyzes the terminal steps of oxidative phosphorylation and its inhibition might be attributed to the alternations in the cellular energy metabolism and decreased formation of free energy due to disruption of mitochondrial membrane and thus interfere with the conversion of oxidative energy to phosphate bond energy. Inhibition of Mg^{2+}-ATPase in the present study suggests prevailed energy crisis, as a result several energy dependant processes such as neural Na^+- K^+ pumps and Ca^{2+}-pumps also disrupted resulting in cellular dysfunction (Cutkomp *et al*., 1982).

Na^+-K^+-ATPase plays a crucial role in active transport of ions across the cell membranes and release of neurotransmitters. Thus reduced activity of Na^+-K^+-ATPase activity in the present study could affect electrical activity of neurons and impairment of synaptic transmission during fenvalerate-induced stress. In support of our findings significant inhibition of Na^+-K^+-ATPase and Mg^{2+}-ATPase was recorded in fish following cypermethrin intoxication (Begum, 2009).

As a corollary to inhibition of ATPase system, crabs might have invoked the phosphatases as an alternate route to mitigate the prevailed energy crisis. In support of our findings, Sreenivasan *et al*. (2009 and 2011) also reported elevation of phosphatases in freshwater field crab, *Spiralothelphusa hydrodroma,* following Cypermethrin toxicity. Increased acid phosphatase activity suggested increased glycogenolysis during fenvalerate-induced stress and enhanced breakdown of phosphates to release energy as a consequence of impaired ATPase system (Reddy *et al.,* 1983)

The phosphatases, because of their cytoplasmic localization and also being non specific towards substrate preference appear to compensate energy by cleaving phosphate esters. In the present study, crabs might have shifted from normal ATPase catabolic route to phosphate system to compensate the prevailed energy crisis. Moreover, the time of peak inhibition of ATPase system (12h) and peak elevation of phosphatases (24h) further reiterated the role of phosphatase system in restoring normalcy through alternate source due to inhibition of ATPase system. The results also corroborates with the biodegradability of pyrethroid compounds in general and fenvalerate in particular. If used judiciously, fenvalerate at lower doses would not produce deleterious effects in non-target animals.

REFERENCES

Begum, G. 2009. Enzymes as biomarkers of cypermethrin toxicity: Response of *Clarias batrachus* tissues ATPase and glycogen phosphorylase as a function of exposure and recovery at sublethal level. *Toxicol. Mech. Methods*. **19:** 29-39.

Bodansky, J. 1932. Determination of inorganic phosphate. Beer's laws and interfering substance in the Kuttner Lichtenstein method. *J. Biol. Chem*. **99:** 197-206.

Bradberry, S. M., Cage, S. A., Proudfoot, A. T. and Vale, J. A. 2005. Poisoning due to pyrethroids**.** *Toxicol. Rev*. **24:** 93-106.

Cutkomp, L. K., Koch, R. B., and Desaiah, D. 1982. Inhibition of ATPases by chlorinated hydrocarbons. In: Insecticide mode of action (J.R Coats, Ed) Academic Press, New York. pp. 45-49.

Desaiah, D., and Ho I. K. 1979. Effects of acute and continuous morphine administration on catecholamine-sensitive adenosine triphosphatase in mouse brain. *J. Pharmacol. Exp. Ther*. **208:** 80-85.

Finney, D. J. 1971. Probit Analysis. Third edition, Cambridge University press, London, p. 333.

Fritz, P. J. and Hamrick, M. E. 1966. Enzymatic analysis of adenosine triphosphatase. *Enzymologia*. **30:** 57-64.

Hill, I. R. 1985. Effects on non-target organisms in terrestrial and aquatic environments. In: The pyrethroid insecticides (J.P. Leahey, Ed) Taylor and Francis, Philadelphia. pp. 151-262.

Koprucu, K. and Aydin, R. 2004. The toxic effects of pyrethroid deltamethrin on common carp (*Cyprinus carpio L.)* embryos and larvae. *Pesticide Biochem. Physiol*. **80:** 47- 53.

Lowry, O. H. and Lopez, J. A. 1946. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem*. **162:** 421-428.

Phillips, T. D. and Hayes, A. W. 1977. Effects of patulin on adenosine triphosphatase activities in the mouse. *Toxicol. Appl. Pharmacol*. **42:** 175-187.

Pillai, S. K. and Sinha, H. C. 1978. Statistical Methods for Biological Workers, Ramprasad and Sons, Agra.pp. 40-58.

Rahmi, A., Koprucu, K., Mustafa, D., Sibel Simsek, K. and Murat, P. 2005. Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio* L) embryos and larvae. *Aquaculture International*. **13**: 451-458.

Reddy, P. S., Bagyalakshmi, A. and Ramamoorthy, R. 1983. Chronic sumithion toxicity: effect on carbohydrate metabolism in crab muscle. *Toxic. Lett*. **22:** 299-309.

Sreenivasan, R. S., Moorthy, P. K. and Deecaraman, M. 2011. Cypermethrin induced toxicity to phosphatases and dehydrogenases in gills and hemolymph of freshwater field crab, *Sipralothelphusa hydrodroma* (Herbst.). *Int. J. Biol. Med. Res*. **2:** 784-788.

Sreenivasan, R. S., Arivalagan, M., Vijayananth, S., Praveenkumar, P. K., Moorthy, P. K. and Deecaraman, M. 2009. Effect of cypermethrin a pyrethroid compound on the neurosecretory cells in the freshwater field crab *Sipralothelphusa hydrodroma* (Herbst.). *Research J. Agriculture and Biological Sciences*. **5:** 566-571.

Weston, D. P., Asbell, A. M., Hecht, S. A., Scholz, M. L. and Lydy, M. J. 2011. Pyrethroid insecticides in urban salmon stream of Pacific North West. *Environ. Pollut*. **159:** 3051-3056.