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In Vivo Inhibition of AChE and ATPase Activities in the Tissues of Freshwater Fish, *Cyprinus carpio* Exposed to Technical Grade Cypermethrin

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Pyrethroid insecticides are extensively used in agriculture because of their desirable properties like high photostability, non-persistent nature and low mammalian toxicity (Casida et al. 1983). Entry of these pyrethroids into the natural aquatic environment causes great havoc to the non-target organisms like fish (David and Somasundaram 1985; Clark et al. 1985). Alteration in the chemical composition of the natural aquatic environment usually affects the biosystem (O'Brien 1967).

Among the synthetic pyrethroids, cypermethrin is one of the potent insecticides (Casida et al. 1983). ATPases are known to play a strategic role in regulating oxidative phosphorylation, ionic transport and several other membrane transport phenomena. Pyrethroids are known to induce toxic effects by the disruption of the nerve impulse transmission and also in modulating neurotransmitter system (Casida et al. 1983). There is no literature available on the toxic impact of cypermethrin on AChE and ATPase activities in freshwater fish, Cyprinus carpio. With all these views, and as the biochemical mode of action of cypermethrin is not known, it seemed important for us to analyse the acute toxicity of cypermethrin on acetylcholine (ACh) level and the activities of AChE, Mg^{2+} ATPase and Na^{+} -K^{+} ATPase in the selected tissues of Cyprinus carpio. This fish was selected as an experimental animal because of its wide occurrence in local ponds and tanks of our area and because it also serves as a rich source of protein for the vast population of Andhra Pradesh, India.

MATERIALS AND METHODS

Male Cyprinus carpio weighing 8±2g were obtained from local fisheries department at Anantapur and were acclimated to laboratory conditions for 15 d. Water used in the present study contained dissolved oxygen of 6-7 ppm; pH 7.4-7.6; temperature 29±1°C; total

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hardness 160 ppm as $CaCO_3$ and total alkalinity 87 ppm as $CaCO_3$. Fish were fed with commercial fish feed and the water was changed daily. Technical grade (96%) cypermethrin (RS) cyano (3-phenoxy phenyl) methyl (1RS) cis-trans-3 -(2,2 dichloroethyl)-2,2-dimethyl cyclopropane carboxylate was obtained from Bharat Pulverising Mills Private Ltd., India. A stock solution of cypermethrin was made in ethanol. The lethal concentration (LC_{50}) for 48 hr to Cyprinus carpio was found to be 60 µg/L (Malla Reddy 1990) which was calculated by the probit method (Finney 1971). One third of the LC_{50} concentration (20 µg/L) was selected as sublethal concentration and used in the present study.

Fish in batches of six each were exposed to sublethal concentration of toxicant (20 µg/L) for 6, 12, 24 and 48 hr. The troughs containing the fish were aerated to prevent hypoxic or anoxic conditions in the medium. No mortality was recorded throughout the exposure period. The animals were killed after 6, 12, 24 and 48 hr of exposure with a blow to the head and tissues namely gill, brain, liver and muscle were isolated and transferred to cold fish Ringer solution which was prepared following the composition given by Ekenberg (1958) and used for different estimations.

ACh content was determined by the method of Hestrin (Augustinson 1957). 5% homogenates of gill, liver, muscle and 1% homogenate of brain were prepared in 0.25M ice cold sucrose solution. The contents were centrifuged at 3000 g at $4^{o}C$ and the supernatants were used for the estimation of AChE (Metacalf 1957). The reaction mixture for AChE contained 1 mL of 0.1M phosphate buffer (pH 7.2) and 0.5 mL of supernatant. For assay of ATPase, 2% homogenates of all tissues were prepared in 0.25M ice cold sucrose solution and centrifuged at 3000 g for 10 minutes at $4^{o}C$ to remove cell debris. The clear cell free extract was used for estimation of Mg^{2+} ATPase and Na^{+}-K^{+} ATPase(Tirri et al. 1953). The reaction mixture of Mg^{2+} ATPase contained 100 µmoles of Tris ATP (0.1M pH 7.4), 25 µmoles of $MgCl_2$, 20 µmoles of NaCl, KCl, 8 µmoles of disodium salt of ATP, 10 µmoles of ouabain and 0.5 mL of extract. The reaction mixture of total ATPase contained 100 µmoles of Tris ATP, 25 µmoles of NaCl, KCl, 8 µmoles of disodium salt of ATP, 20 µmoles of $MgCl_2$ and 0.5 mL of extract. The inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). The Na^{+}-K^{+} ATPase activity was calculated by substracting the Mg^{2+} ATPase from the total ATPase activity. Protein in the tissues was estimated by the Folin phenol method (Lowry et al. 1951) using bovine serum albumen as standard.

The average of six individual estimations was taken and the mean values of control and experimental fishes were subjected to statistical treatment using analysis of variance (ANOVA) as described by Pillai and Sinha (1968). If the difference between control and experimental values exceeds the critical difference (C.D.), the values were considered as significant at 5% level.

RESULTS AND DISCUSSION

The activity of AChE was inhibited with an elevation of ACh content in all the tissues, viz., gill, brain, liver and muscle at all periods of exposure to 20 µg/L of technical grade cypermethrin (Tables 1-2). All the values were statistically significant at a 5% level except the decrease in ACh content at 6 hr exposure in liver and gill tissues. Brain tissue was affected to a greater extent when compared to other three tissues.

The observed inhibition of AChE in the present investigation was in agreement with the findings of Bandyopadhyay (1982) who reported a significant decrease of this enzyme in brain of rat treated with permethrin. Increased AChE inhibition with increase in exposure time seen in this study may be interpreted as a change due to cumulative action of cypermethrin.

The other neural tissue, muscle and gill also exhibited a greater inhibition of AChE enzyme indicating alterations on the biochemical processes thereby influencing the ACh metabolism. Earlier studies of AChE activity in the tissues of Periplaneta americana exposed to fenvalerate disclose the same trend observed in the present investigation (Yellamma and Ravikumar Reddy 1987). Inhibition of this enzyme in the liver may be due to the synergistic action of the parent compound and its metabolites which are formed as a result of biodegradation (Casida et al. 1983).

Both Mg^{2+} and Na^{+}-K^{+} dependent ATPases were inhibited in all the tissues of fish exposed to cypermethrin (Tables 3-4). All the changes were significant at 5% level except the decrease in Na^{+}-K^{+} ATPase activity at 6 hr exposure in liver and muscle tissues.

Mg^{2+} ATPase enzyme is found in association with both Na^{+}-K^{+} and Na^{+}-NH_4^{+} ATPase in fishes and is related to the transport of Mg^{2+} across the gill epithelium and is also essential for the integrity of the cellular membrane and for the stabilization of branchial permeability (Isai and Masoni 1976). Na^{+}-K^{+} ATPase is an important component of active transport in teleost gills and it is a biochemical expression of active transport of Na^{+} and K^{+} in the cells (Skou 1975).

Table 1. Alterations in acetylcholine content (µmoles of ACh/g tissue) in the tissues (wet wt) of fish, Cyprinus carpio exposed to 20 µg/L of cypermethrin

Tissue	Control	Experimental	Hours of exposure	Percent change over control	Level of significance
Brain	54.93 ±1.06	62.48±5.24	6	+13.7	
		79.26±7.48	12	+44.3	F=1192.98
		91.46±5.68	24	+66.5	CD=6.86
		102.2+10.56	48	+86.0	
Liver	15.96 ±1.82	17.36±1.62*	6	+ 8.8	
		21.45±1.10	12	+34.4	F=569.04
		24.14±2.77	24	+51.2	CD=2.94
		26.93±3.97	48	+68.7	
Gill	26.17 ±3.34	30.03±5.15*	6	+14.7	
		31.87±3.68	12	+21.8	F=618.04
		38.15±3.06	24	+45.8	CD=4.44
		41.09±2.99	48	+57.0	
Muscle	28.31 ±1.22	32.7±4.09	6	+15.5	
		36.1±3.07	12	+27.6	F=1271.71
		40.6±3.31	24	+43.3	CD=3.49
		49.8±2.10	48	+75.9	

Each value is mean ± SD (n=6); F=Variance ratio; CD= Critical difference; * denotes not significant at 5% level.

The results obtained in the present study indicate the possibility of disruption due to the effect of cypermethrin on passive movement of ions i.e., the permeability characteristics. In this connection, it is of interest to note that O_2 consumption decreased at whole animal and at tissue levels in this test animal exposed to sublethal concentration of cypermethrin (Malla Reddy 1990) and also exhibited the inhibition of oxidative metabolic enzymes like isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and cytochrome-c-oxidase in the fish, Labeo rohita exposed to cypermethrin (Ghosh 1989). The decrease of these two enzymes may be correlated with a low availability of substrate as reported by McKee and Knowles (1986) in Daphnia magna exposed to fenvalerate.

In support to the findings of the present investigation earlier reports also showed a decrease in Ca^{2+}+Mg^{2+} ATPase activity in the squid, Loligo pealei (Clark and Matsumura 1987) exposed to wide variety of pyrethroids. Inhibition of Mg^{2+} ATPase activity in Labeo rohita exposed to cypermethrin (Ghosh 1989), and the ATPase

Table 2. Alterations in the AChE activity (µmoles of ACh hydrolysed/mg protein/hr) in the tissues (wet wt) of fish, Cyprinus carpio exposed to 20 µg/L of cypermethrin

Tissue	Control	Experimental	Hours of exposure	Percent change over control	Level of significance
Brain	8.94 ±0.16	7.51±0.07	6	-15.2	
		6.77±0.07	12	-24.3	F=22204.7
		5.25±0.18	24	-41.3	CD=0.15
		3.50±0.08	48	-60.8	
Liver	2.93 ±0.11	2.74±0.05	6	- 6.5	
		2.59±0.07	12	-11.6	F=8038.7
		2.30±0.06	24	-21.5	CD=0.09
		2.03±0.08	48	-30.7	
Gill	4.49 ±0.31	4.11±0.08	6	- 8.5	
		3.85±0.05	12	-14.2	F=3939.5
		3.32±0.08	24	-26.1	CD=0.19
		2.82±0.04	48	-37.2	
Muscle	5.31 ±0.14	4.85±0.14	6	- 8.7	
		4.42±0.17	12	-16.8	F=8054.2
		3.89±0.08	24	-26.7	CD=0.16
		3.19±0.05	48	-39.9	

Each value is mean ± SD (n=6); F=Variance ratio; CD=Critical difference; All values are significant at 5% level.

system of Cyprinus carpio under fenvalerate stress (Malla Reddy et al. 1991) also exemplify the results obtained in our experiments.

In freshwater fishes, Mg^{2+} and Na^{+}-K^{+} ATPases play a significant role in ionic regulation and aid in salt uptake from the ambient medium (Pfeiler and Kirschner 1972). Hence, inhibition of these activities in the gill of the fish exposed to cypermethrin indicates disruption in its cellular and ionic regulation and salt uptake for the pyrethroids are effeciently absorbed across gills (Bradbury et al. 1987).

ATPases actively participate in the transmission of nerve impulse. Hence, the low activities of these ATPases in brain of the fish exposed to cypermethrin suggests a decrease in the transmission of nerve impulse. Liver, being an important centre for metabolism, interconversion and storage of food stuffs, also exhibited reduced activities of ATPases during cypermethrin exposure. This suggests possible

Table 3. Alterations in the Mg^{2+} ATPase activity (µmoles of pi formed/mg protein/hr) in the tissues (wet wt) of fish, Cyprinus carpio exposed to 20 µg/L of cypermethrin

Tissue	Control	Experimental	Hours of exposure	Percent change over control	Level of significance
Brain	5.07	4.63±0.14	6	- 8.7	
	±0.11	3.75±0.14	12	-26.0	F=9161.5
		2.88±0.05	24	-43.2	CD=0.13
		1.93±0.08	48	-61.9	
Liver	6.47	5.51±0.14	6	-14.8	
	±0.16	4.35±0.13	12	-32.8	F=10496.5
		3.14±0.08	24	-51.5	CD=0.15
		1.94±0.07	48	-70.0	
Gill	4.27	3.83±0.15	6	-10.3	
	±0.21	3.18±0.09	12	-25.5	F=4860.6
		2.76±0.08	24	-35.4	CD=0.16
		2.10±0.08	48	-50.8	
Muscle	5.48	4.85±0.05	6	-11.5	
	±0.11	3.91±0.07	12	-28.6	F=17729.3
		3.08±0.08	24	-43.8	CD=0.09
		2.08±0.08	48	-62.0	

Each value is mean ± SD (n=6); F=Variance ratio; CD=Critical difference; All values are significant at 5% level.

deformities in ionic regulation of its cellular components thus causing disturbances and metabolic diversions.

As ATPases are intimately associated with the synaptic transmission at the neuromuscular junctions, the decrease in the activities of ATPases in muscle of the fish observed in the present study probably causes an imbalance in a corresponding level of synaptic transmission at the neuromuscular junction during the period of toxic stress.

The observations made here clearly indicate that cypermethrin is a potent inhibitor of both AChE and ATPase activities. But this inhibitory pattern however should be further verified by enzyme purification studies and kinetics to yield insight into the exact point of interference. Extension of these studies under conditions of chronic exposure would reveal the intimate nature of cypermethrin toxicity.

Table 4.Alterations in the Na^+-K^+ ATPase activity (µmoles of pi formed/mg protein/hr) in the tissues (wet wt) of fish, Cyprinus carpio exposed to 20 µg/L of cypermethrin

Tissue	Control	Experimental	Hours of exposure	Percent change over control	Level of significance
Brain	7.09 ±0.19	6.61±0.64	6	- 6.8	
		5.49±0.21	12	-22.6	F=2223.9
		4.91±0.13	24	-30.7	CD=0.40
		4.54±0.25	48	-35.9	
Liver	2.85 ±0.79	2.70±0.36*	6	- 5.3	
		2.44±0.23	12	-14.4	F=282.9
		2.32±0.15	24	-18.5	CD=0.49
		2.15±0.07	48	-24.7	
Gill	8.53 ±0.44	7.82±0.16	6	- 8.3	
		6.25±0.31	12	-26.7	F=4237.9
		5.57±0.22	24	-34.7	CD=0.34
		4.97±0.19	48	-41.7	
Muscle	3.85 ±0.45	3.63±0.19*	6	- 5.7	
		3.16±0.23	12	-17.9	F=1093.0
		2.91±0.17	24	-24.4	CD=0.34
		2.82±0.12	48	-26.7	

Each value is mean ± SD (n=6); F=Variance ratio; CD=Critical difference; * denotes not significant at 5% level.

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