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Effect of Endosulfan on Adenosine Triphosphatase (ATPase) Activity in Liver, Kidney, and Muscles of *Channa gachua*

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Large scale application of pesticides to agricultural and forest areas may contribute to the presence of these toxic substances in the environment. Among these different kinds, that of organochlorines require special attention because of the high stability and toxicity these compounds display with regard to aquatic flora and fauna. Toxicity of these compounds to aquatic organisms is hundred times greater than that of organophosphorus compounds (Rodier 1978).

The present study was undertaken to determine the effect of an organochlorine insecticide, Endosulfan (6, 7, 8, 9, 10, 10-hexachloro- 1, 5, 5a, 6, 9, 9a -hexahydro- 6, 9, -methano - 2, 4, 3-benzo dioxathiepin 3-oxide) on the activity of adenosine triphosphatase (ATPase) in liver, kidney and muscles of a freshwater teleost, Channa gachua.

MATERIALS AND METHODS

Healthy fish Channa gachua were collected in March 1987 from River Yamuna (New Delhi) and transported immediately to the laboratory where they were transferred into 200-L aquaria. They were maintained for 15 days at 22±2°C in tap water and were fed a daily diet of pork liver. Some of the chemical characteristics of the water used are given in Table 1.

Then, 60 fish ranging in weight from 15.5 to 21.7 g and fork length 11.2 to 14.5 cm were divided into three groups of 20 animals each. They were kept in 25-L experimental aquaria containing tap water. The endosulfan stock solution of 1 g/L of water was prepared. Aliquots of this stock solution were added to each test aquaria to bring the endosulfan concentrations to the desired levels of 0.0022, 0.0037 and 0.0056 mg/L. All aquaria were kept at a constant temperature. The water was changed daily to reduce the build up of metabolic wastes and to keep concentrations of endosulfan near the nominal level. 20 more fish served as a control and were kept in clean water. After 15 and 30 days of exposure the animals were transferred to clean water and liver, kidney and muscles (red muscles from

anterior region) of control and treated fish were removed. Enzyme activity was measured using the method of Pullman et al (1960). The tissue homogenate was prepared in ice-cold 0.32 M sucrose, 1.0 mM disodium ethylenediaminetetraacetic acid and 10 mM imidazol buffer at pH 7.5. The homogenate was spun at 900 x g in a refrigerated centrifuge for 10 min and the supernatant at 1300 x g for 12 min. The sediments were suspended in cold 0.32 M sucrose solution.

Total ATPase was measured when Na^+, K^+ and Mg^{2+} were present in the reaction mixture. Mg^{2+} ATPase activity was measured in presence of 1.0 mM ouabain, a specific inhibitor of Na^+, K^+ ATPase (Matsumura et al. 1969). Mg^{2+} ATPase activity was delineated into oligomycin-insensitive Mg^{2+} ATPase (OIS Mg^{2+} ATPase) by adding 5×10^{-6} M oligomycin in 1.0 µL ethanol.

Table 1. Range of chemical characteristics of the tap water used for experimentation

Parameters	Values*
pH	7.5 - 7.7
Dissolved oxygen	7.2 - 7.9
Total Hardness	55 - 71
Total solids	33.5 - 34.9
Solids (volatile)	13.2 - 14.1
Silica	2.1 - 2.4
Nitrogen	0.15 - 0.21
Chloride	1.2 - 3.5
Phosphate	0.1 - 0.5
Sulphate	4.2 - 5.7

* All values except pH are in mg/L

Oligomycin is a specific inhibitor for the mitochondrial portion of the total Mg^{2+} ATPase activity (Lardy et al. 1964).

Reaction mixture used for the assay was as follows: 4.5 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazol buffer (pH 7.5), 0.2 mM NADH, 0.5 mM phosphenol pyruvate, 0.02% bovine serum albumin, 9 units of pyruvate kinase, 12 units of lectate dehydrogenase and 100 µL of tissue homogenate as enzyme source. Absorbance was measured at 340 nm using Spectronic 1001 Spectrophotometer.

The inorganic phosphate liberated was estimated by the method of Fiske and Subba Rao (1925). Enzyme activity is expressed as ug of inorganic phosphate liberated/h/mg of protein. Protein was estimated according to Lowry et al (1951) using bovine serum albumin (BSA) as the standard. All other chemicals used were of the best available reagent grade and were purchased from Sigma Chemical Co., St. Louis, Missouri, USA.

Table 2. ATPase activity in liver of Channa gachua after 15 and 30 days of exposure to different concentrations of endosulfan

Time (days)	Concentration (mg/L)	Enzyme activity = µg inorganic phosphate liberated/h/mg protein		
		Na^+, K^+ATPase[a]	Mg^{2+} ATPase[a]	
			Oligomycin sensitive	Oligomycin insensitive
15	Control	0.755 ± 0.012	0.440 ± 0.014	1.490 ± 0.120
	0.0022	0.813 ± 0.015 (7.8)[b]	0.404 ± 0.017 (8.1)	1.510 ± 0.120 (1.2)[b]
	0.0037	0.539 ± 0.021 (28.6)***	0.367 ± 0.011(16.6)	1.170 ± 0.110 (21.8)
	0.0056	0.485 ± 0.016 (35.8)***	0.297 ± 0.013(32.6)*	0.950 ± 0.140 (36.1)*
30	Control	0.708 ± 0.010	0.400 ± 0.013	1.390 ± 0.100
	0.0022	0.596 ± 0.017 (15.8)*	0.332 ± 0.010 (17.0)*	1.230 ± 0.140 (11.8)
	0.0037	0.474 ± 0.016 (33.1)***	0.306 ± 0.010 (23.6)*	1.030 ± 0.160 (25.8)
	0.0056	0.364 ± 0.011 (48.6)***	0.249 ± 0.015 (37.8)**	0.850 ± 0.120 (39.5)**

a Mean ± SE; b Percent stimulation; other values in parentheses are percent inhibition; values are significant at - *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Fisher's t test)

Table 3. ATPase activity in kidney of Channa gachua after 15 and 30 days of exposure to different concentrations of endosulfan

Time (days)	Concentration (mg/L)	Enzyme activity = µg inorganic phosphate liberated/h/mg protein		
		Na^+, K^+ ATPase[a]	Mg^{2+} ATPase[a]	
			Oligomycin sensitive	Oligomycin insensitive
15	Control	1.940 ± 0.120	0.778 ± 0.013	0.156 ± 0.014
	0.0022	2.010 ± 0.130 (3.4)[b]	0.678 ± 0.012 (12.8)**	0.143 ± 0.012 (8.6)
	0.0037	1.400 ± 0.160 (27.8)	0.579 ± 0.014 (25.6)***	0.125 ± 0.016 (19.9)
	0.0056	1.280 ± 0.170 (33.8)*	0.539 ± 0.016 (30.7)***	0.112 ± 0.012 (28.4)
30	Control	1.930 ± 0.100	0.771 ± 0.010	0.148 ± 0.015
	0.0022	1.760 ± 0.100 (8.9)	0.680 ± 0.016 (11.8)**	0.128 ± 0.11 (13.4)
	0.0037	1.370 ± 0.100 (28.8)*	0.560 ± 0.011 (27.3)***	0.115 ± 0.010 (22.3)
	0.0056	1.240 ± 0.100 (35.8)	0.520 ± 0.010 (32.6)***	0.098 ± 0.010 (34.0)*

a Mean ± SE; b Percent stimulation; other values in parentheses are percent inhibition; Values are significant at - *P < 0.05; **P < 0.01; ***P < 0.001 (Fisher's t test)

Table 4. ATPase activity in the muscles of Channa gachua after 15 and 30 days of exposure to different concentrations of endosulfan

Time (days)	Concentration (mg/L)	Enzyme activity = µg inorganic phosphate liberated/h/mg protein		
		Na^+, K^+ ATPase[a]	Mg^{2+} ATPase[a]	
			Oligomycin sensitive	Oligomycin insensitive
15	Control	0.338 ± 0.012	0.790 ± 0.015	0.162 ± 0.011
	0.0022	0.300 ± 0.010 (11.2)	0.714 ± 0.012 (9.6)**	0.157 ± 0.010 (0.33)
	0.0037	0.255 ± 0.011 (24.6)**	0.623 ± 0.017 (21.1)**	0.130 ± 0.010 (19.8)
	0.0056	0.207 ± 0.011 (38.8)**	0.508 ± 0.014 (35.6)***	0.112 ± 0.010 (31.3)*
30	Control	0.329 ± 0.011	0.760 ± 0.012	1.680 ± 0.181
	0.0022	0.284 ± 0.011 (13.8)*	0.670 ± 0.012 (11.8)*	1.480 ± 0.160 (6.6)
	0.0037	0.235 ± 0.012 (28.6)	0.568 ± 0.017 (25.3)***	1.240 ± 0.107 (21.7)
	0.0056	0.178 ± 0.011 (45.8)***	0.470 ± 0.016 (38.2)**	1.050 ± 0.101 (33.8)

a Mean ± SE; other values in parentheses are percent inhibition; values are significant at *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Fisher's t test)

RESULTS AND DISCUSSION

The inhibition of ATPase activity was in the order of liver > muscles > kidney (Table 2-4). In liver, the Na^+, K^+, ATPase activity was significantly ($P < 0.001$) inhibited at 0.0056 mg/L whereas oligomycin-sensitive Mg^{2+} ATPase (OS Mg^{2+} ATPase) was found to be inhibited significantly ($P < 0.01$) in kidney and muscles. Oligomycin-insensitive Mg^{2+} ATPase (OIS Mg^{2+} ATPase) activity was significantly ($P < 0.05$) inhibited in liver and muscles after 15 days exposure to different concentrations of endosulfan (Table 2 and 4). However, after 15 days of exposure time, the Na^+, K^+ ATPase activity was found to be activated in liver and kidney at the lowest concentration of endosulfan used (0.0022 mg/L). A slight increase in OIS Mg^{2+} ATPase activity in liver was observed after 15 days of exposure at 0.0022 mg/L endosulfan (Table 2).

Maximum inhibition of ATPase was observed in liver Na^+, K^+ ATPase at 0.0056 and 0.0037 mg/L concentration after 15 and 30 days exposure, respectively. After 30 days exposure, the Na^+, K^+ ATPase activity was significantly ($P < 0.001$) inhibited in liver followed by muscles at 0.0056 mg/L. OS Mg^{2+} ATPase activity was significantly ($P < 0.001$) and highly inhibited in kidney at 0.0056 and 0.0037 mg/L of endosulfan followed by muscles at 0.0037 mg/L (Table 3). In the present study it is seen that, in general, OIS Mg^{2+} ATPase was not nearly as sensitive to endosulfan as OS Mg^{2+} ATPase. The high sensitivity of OS Mg^{2+} ATPase activity was also observed by Desaiah et al (1977 a;b, 1979) and Desaiah (1980) in rat and by Yap et al (1975) in bluegill brain. Endosulfan, like organotin compound cyhexatin (Plictran) is highly effective as an inhibitor of OS Mg^{2+} ATPase but, unlike DDT, is also highly inhibitory of Na^+, K^+ ATPase as well (Desaiah et al. 1973). Na^+, K^+ ATPase shows high sensitivity to endosulfan than Mg^{2+} ATPase in the present study. Toxaphene has been shown to have a greater effect on Na^+, K^+ ATPase than on Mg^{2+} ATPase in the cockroach central nervous system and in kidney homogenate from mice (Fattah and Crowder 1980).

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