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Effect of Endosulfan, Triphenyltin Acetate, Thiodicarb and Lambda-Cyhalothrin on the Activity of Some Enzymes in Cat Fish (Ictalurus punctatus) Tissues

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ABSTRACT

The effects of four pesticides on the activity of six enzymes were examined in vitro in different tissues of the cat fish, Ictalurus punctatus. The ATPase activity in brain and muscle was decreased by endosulfan, λ-cyhalothrin, and triphenyltin acetate. Thiodicarb did not show specific affinity to ATPase. The acetylcholinesterase (AChE) activity in brain was inhibited by thiodicarb, whereas endosulfan and λ-cyhalothrin caused much less inhibition. Triphenyltin acetate did not show any interaction with acetylcholinesterase (AChE), acid phosphatase (AcP), alkaline phosphatase (AlP), glutamate-oxaloacetate transaminase (GOT), and glutamate-pyruvate transaminase (GPT). The hepatic AcP in kidney was inhibited by endosulfan, thiodicarb and λ-cyhalothrin. In the liver, however, the AcP activity was generally enhanced. The AlP activity was increased by endosulfan and thiodicarb in the kidney. In the liver, the AlP activity was decreased with endosulfan and thiodicarb. The GOT was inhibited in the kidney and liver by endosulfan and thiodicarb. The GPT activity was increased by two pesticides in the liver and muscle. λ-cyhalothrin did not show any interaction with AlP, GOT and GPT.

INTRODUCTION

The extensive use of pesticides in agriculture has given rise to criticism in recent years. Several pesticides have persistent nature in the environment and accumulation in different tissues of animals and human beings.

In recent years, many investigators have studied the effects of pesticides on several enzyme activities

in fish tissues, such as acid and alkaline phosphatases (Gill et al., 1990), hexokinase (Sastry and Siddiqui, 1983), transaminase (Christensen et al., 1982), ATPase (Cutkomp et al., 1971), and lactic dehydrogenase (Natarajan, 1984). Many pesticides are potential antiacetylcholinesterase agents (carbamates and organophosphorus compounds), O'Brien, 1961. Pesticide-induced alterations in the enzyme activities, may, have diagnostic significance in the evaluation of adverse health effects of toxic substances.

The goal of this investigation was to study the specific activity of six different enzymes (ATPase, AChE, AcP, AlP, GOT, and GPT) in different tissues of cat fish and to determine their sensitivity to a chlorinated hydrocarbon insecticide (endosulfan), an organotin fungicide (triphenyltin acetate), a carbamate insecticide (thiodicarb), and a pyrethroid insecticide (λ-cyhalothrin).

MATERIALS AND METHODS

Source of Chemicals

Endosulfan and thiodicarb were standard analytical samples from the Environmental Protection Agency (EPA), Research Triangle Park, N.C., U.S.A. Triphenyltin acetate and λ-cyhalothrin were the kind gifts of Prof. R.B. Beechey, University College of Wales, Aberystwyth, U.K. All other chemicals were obtained from Sigma Company, Poole, Dorset, U.K.

Source of Tissues

Cat fish Ictalurus punctatus ranging between 150-200 g/individual were collected from fresh water canals at "Motobus" in Kafr El-Sheikh Governorate. The fish were kept in large tanks in aerated tap water and left to acclimatize to laboratory conditions for about four weeks before the experiment began.

Enzymes preparation:

Adenosine triphosphatase (ATPase)

Mitochondria preparations from cat fish tissues were prepared by the method of Koch (1969). Tissues from cat fish were dissected and homogenized in ice cold buffer 0.25 M sucrose, 1 mM EDTA and 20 mM Tris-HCl pH 7.4. The resulting homogenate was filtered through a

double layers of cheese cloth, then centrifuged at 900 xg for 10 min at 4°C. The supernatant was then recentrifuged at 13,000 xg for 20 min at 4°C. The pellets (mitochondria) were washed twice with 0.25 M sucrose pH 7.5 and finally suspended in the same buffer to be adjusted to the appropriate protein concentration.

ATPase assay

Total ATPase assays were carried out at 37°C in a medium containing 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl pH 8.0, 5 mM ATP, and an enzyme preparation (15–25 µg) in a final volume of 1 ml. After 30 min, the reaction was stopped by adding 100 µl of cold 50% trichloroacetic acid. ATPase activity was expressed as µmoles Pi released.mg^{-1}protein.min^{-1}. Pi was assayed according to the method of Taussky and Shorr, 1953. Mg^{2+}-ATPase activity was measured when 0.85 mM ouabain (the specific inhibitor for Na^+-K^+-ATPase) was in the mixture. Na^+-K^+-ATPase activity is total activity minus the Mg^{2+}-ATPase activity.

AChE, AcP, AlP, GOT and GPT

Liver, brain, muscles and kidney were removed from each fish and quickly weighed. Tissues were homogenized immediately in cold 0.25 M sucrose at the following dilutions (w/v): brain 1%, kidney and liver 6% and muscles 10%. The homogenates were centrifuged at 3000 xg for 10 min, and the clear supernatant was used for measurement of enzyme activities. The AChE was estimated by the method of Ellman et al., 1961. AcP and AlP were determined according to Bergmeyer (1963). GOT and GPT were assayed according to Reitman and Frankel (1957), using a commercially available kit from Bio Merieux. Protein was determined by the method of Lowry et al., 1951, using bovine serum albumin as standard.

For inhibition studies, endosulfan and triphenyltin acetate were prepared in ethanol as 10 mM stock solutions. Solutions of λ-cyhalothrin and thiodicarb were prepared in acetone as 10 mM stock solutions. For all inhibitors used appropriate controls were run. Reaction blanks were prepared for each treatment and subtracted to calculate the enzyme activity.

RESULTS AND DISCUSSION

Enzyme activities of cat fish tissues

Specific activities of four different tissues from cat fish (Table 1) show that brain homogenate was highest in Na^+-K^+-ATPase and AChE. Mg^{2+}-ATPase and GPT were most active in muscle. Kidney preparation had a very high Na^+-K^+-ATPase and AlP activities. Liver had a high AcP and GOT activities. These findings are in general agreement with earlier reports in other fishes (Coppage et al., 1975, Gill et al., 1990).

Effect of pesticides on some enzymes in different tissues of cat fish:

The aim of the following experiments were to evaluate the effect of four pesticides on the enzymes selected for this study, and are represented in Tables 2 to 4.

Effects on ATPase activity

The data illustrated in Table 2 show that endosulfan concentrations (5, 50, 100 µM) were inhibitory to Na^+-K^+-ATPase and Mg^{+2}-ATPase in both brain and muscle homogenates. A 40% inhibition of the ATPase activity was shown with 50 µM endosulfan. This finding is in agreement with the results reported by several researchers (Koch, 1969 and Cutkomp et al., 1971).

Triphenyltin acetate a potent inhibitor of oxidative phosphorylation (Byington, 1971) was found to strongly inhibit the activity of the Mg^{2+}-ATPase of muscle cat fish (Table 2). The percentage of inhibition ranged from 22-75%) with triphenyltin acetate concentrations (5-100 µM). These results were consistent with results previously reported by Byington (1971) on the adenosine triphosphatase activity of beef heart submitochondrial particles, and Rose and Aldridge (1972) on mitochondrial ATPase from rat liver.

The effect of λ-cyhalothrin on Mg^{2+}-ATPase from brain and muscle of cat fish is shown in Table 2. The enzyme was highly sensitive to the action of λ-cyhalothrin under in vitro conditions. A 78% inhibition of the enzyme activity was shown with both the 2 and 20 µM concentrations. The power of inhibition of λ-cyhalothrin could be compared to that of oligomycin (a well known specific inhibitor of Mg^{2+}-ATPase). This finding is in agreement with

Table 1. Enzyme activities in different tissues of control cat fish.

Enzyme	Brain	Muscle	Kidney	Liver
Mg^{2+}-ATPase (µmole Pi/mg protein/min)	0.32 ± 0.02	0.75 ± 0.04	0.60 ± 0.03	0.40 ± 0.035
Na^{+}-K^{+}-ATPase (µmole Pi/mg protein/min)	0.53 ± 0.012	0.083 ± 0.0015	0.73 ± 0.01	0.05 ± 0.001
AChE (µmoles ATChI/mg protein/min)	53.2 ± 2.4	ND*	ND	ND
AcP (1 U/mg protein)	ND	ND	5.74 ± 0.68	8.5 ± 1.54
AlP (1 U/mg protein)	NB	ND	62.2 ± 1.45	4.3 ± 0.66
GOT (Units/ml)	ND	ND	5.1 ± 0.03	6.2 ± 0.08
GPT (Units/ml)	ND	6.8 ± 0.17	ND	6.0 ± 0.33
LDH (1 U/mg protein)	ND	1622 ± 116	ND	704 ± 48

* ND: not detected
Values are mean ± SE

Table 2. *In vitro* effects of endosulfan, triphenyltin acetate, thiodicarb and λ-cyhalothrin on the ATPase activity in different tissues of cat fish.

Inhibitor	Concentration (μM)	Percent activity of control		
		Brain		Muscle
		Na^+-K^+-ATPase	Mg^{2+}-ATPase	Mg^{+2}-ATPase
None	—	100.0	100.0	100.0
Endosulfan	5	85.3	81.0	73.0
	50	78.2	66.5	60.4
	100	71.0	68.0	62.7
Triphenyltin acetate	5	100.0	92.0	78.0
	50	100.0	80.6	25.2
	100	100.0	76.3	25.2
Thiodicarb	100	97.0	98.0	92.0
λ-Cyhalothrin	2	88.2	21.6	22.0
	20	88.0	22.0	22.0

ATPase assays were carried out as described in "Materials and Methods". Membrane ATPases were preincubated with the specific inhibitor for 5 min at 37°C prior to starting ATPase assay by addition of the reaction mixture.

Table 3. In vitro effects of endosulfan, triphenyltin acetate, thiodicarb and λ-cyhalothrin on AChE, AcP and AlP activities in different tissues of cat fish.

Inhibitor	Concentration (μM)	Percent activity of control				
		Enzymes				
		AChE	AcP		AlP	
		Brain	Kidney	Liver	Kidney	Liver
None	—	100.0	100.0	100.0	100.0	100.0
Endosulfan	50	90.0	88.0	109.0	115.3	72.4
	100	86.4	70.6	117.0	119.0	67.0
Triphenyltin acetate	100	100.0	100.0	98.2	100.0	100.0
Thiodicarb	10	72.3	93.7	106.6	109.2	81.8
	50	31.5	86.0	113.2	116.4	77.8
	100	22.0	81.0	118.0	120.7	73.2
λ-Cyhalothrin	2	96.0	90.0	107.5	100.0	100.0
	20	88.4	83.2	112.0	100.0	100.0

the results reported by Patil et al. (1979) using pyrethrin on Mg^{2+}-ATPase from house flies.

No significant inhibition of the ATPase was observed using 100 µM of thiodicarb, or by triphenyltin acetate and λ-cyhalothrin on the activity of Na^{+}-K^{+}-ATPase. Higher concentrations of all inhibitor did not further inhibit ATPases.

Effects on AChE activity

Thiodicarb caused an inhibition (79%) by 100 µM of AChE activity in the brain (Table 3), whereas endosulfan and λ-cyhalothrin had no effect. Triphenyltin acetate did not inhibit AChE activity under in vitro situation. These findings are in general agreement with the results reported by Gill et al., 1990 and Coppage et al., 1975.

In conclusion, this study pointed out that brain AChE inhibition in fish can be used as a marker for environmental poisoning by thiodicarb.

Effects on AcP activity

In the kidney, a decrease in the AcP activity was noted in vitro by endosulfan, thiodicarb and λ-cyhalothrin (Table 3). In the liver, stimulation of AcP activity occurred in vitro by endosulfan, thiodicarb, and λ-cyhalothrin. On the other hand, triphenyltin acetate did not show any interaction with AcP.

The AcP is a lysosomal enzyme and the rise in its activity is probably related to the cellular damage. The increased AcP activity seems to result from enhanced enzyme turnover under pesticide stress, whereas a decrease may be related to leakage of the enzyme into extracellular compartments. Thus, the results of this study dealing with pesticides-induced modulation of AcP are in agreement with the results reported by Gill et al., 1990.

Effects on AlP activity

Transport of nutrient materials across the intestinal brush border is stated to be facilitated by the AlP. The data represented in Table 3 show that endosulfan and thiodicarb caused an stimulation of AlP activity in the kidney. Effects on AlP activity in the liver included an inhibition by the two pesticides. Triphenyltin acetate and λ-cyhalothrin did not show any interaction with this enzyme.

Table 4. *In vitro* effects of endosulfan, triphenyltin acetate, thiodicarb and λ-cyhalothrin on GOT and GPT activities in different tissues of cat fish.

Inhibitor	Concentration (μM)	Percent activity of control			
		Enzymes			
		GOT		GPT	
		Kidney	Liver	Muscle	Liver
None	—	100.0	100.0	100.0	100.0
Endosulfan	50	84.0	81.0	103.6	107.0
	100	78.0	74.5	110.0	115.4
Triphenyltin acetate	100	98.3	99.2	96.0	100.0
Thiodicarb	10	95.4	90.0	104.4	105.0
	50	88.0	83.0	111.2	113.4
	100	83.3	79.4	118.7	116.2
λ-Cyhalothrin	2	100.0	100.0	100.0	100.0
	20	97.0	95.2	100.0	98.8

The decrease in the AlP activity might be due to tissue damage resulting in an overall insufficient compensatory enzyme production while the enhanced activity could be related to the influence of glucocorticoids, as also reported in the higher vertebra es. The data agrees with the results of Gill et al., 1990.

Effect on transaminases activity

The transaminases, GOT and GPT, are two key enzymes known for their role in the utilization of proteins and carbohydrates.

The results illustrated in Table 4 represent the effect of both endosulfan and thiodicarb on the activity of GOT from liver and kidney of the experimental fish. The two pesticides decreased the GOT activity. The extent of inhibition did not vary much with the class of the chemical used or the concentration tested. Both endosulfan and thiodicarb caused slightly stimulation (15%) in muscle and liver GPT (Table 4). Triphenyltin and λ-cyhalothrin did not enhance or inhibit the activity of GOT and GPT. This finding is in agreement with the results reported by Dalvi, 1988, and Christensen et al., 1982.

The data presented in this paper have an application in the development of a rapid screening procedure involving enzymes as biomonitors in analyzing water quality.

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الملخص العربي

تم تقدير نشاط عدة انزيمات في كبد ، مخ ، كلية ، وعضلات سمك الصلور وهي الأدينوزين ثالث الفوسفاتيز ، الاستيل كولين استريز ،الاسيد فوسفاتيز ، قلوي فوسفاتيز ، الجلوتاميك بيروفيك ترانس امينيز . وقد درس تاثير المبيدات الاتية : الاندوسلفان ، تراي فينايل تين اسيتات ، الثيوداي كارب ، لاندا سايهالوثرين علي هذه النظم الانزيمية . واوضحت النتائج ان . الاندوسلفان وتراي فينايل تين اسيتات ولاندا ـ سايهالوثرين تحدث تثبيطا لنشاط انزيم الادينوزين ثالث الفوسفاتيز في الانسجة المختبرة ولم يظهر اي تاثير لمركب الثيوداي كارب علي نشاط هذا الانزيم . احدث الثيوداي كارب تثبيطا عاليا لانزيم الاستيل كولين استريز اما بقية المركبات فكان التثبيط فيها منخفض ماعدا التراي فينايل تين اسيتات حيث لم يظهر له اي تاثير علي بقية الانزيمات المختبرة وقد احدثت المبيدات المختبرة تثبيطا لنشاط انزيم الاسيد فوسفاتيز في الكلي بينما احدثت زيادة في نشاط هذا الانزيم في الكبد . احدث كل من الاندوسلفان والثيوداي كارب زيادة في نشاط انزيم الجلوتاميك او كسالواسيتات ترانس امينيز وزيادة في نشاط انزيم الجلوتاميك بيروفيك ترانس امينيز بواسطة الاندوسلفان والثيوداي كارب في الانسجة المختبرة . لم تظهر النتائج اي تاثير لمركب لاندا ـ سايهالوثرين علي نشاط انزيم قلوي الفوسفاتيز والجلوتاميك اوكسالواسيتات ترانس امينيز والجلوتاميك بيروفيك ترانس امينيز .