Toxaphene Inhibition of ATPase Activity in Catfish, *Ictalurus punctatus*, Tissues*

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Toxaphene is a very complex mixture of compounds. It has been shown very recently that toxaphene has about 175 C_{10} polychloro derivatives (CASIDA et al. 1974). These authors have also identified 2,2,5-endo, 6-exo, 8,9,10-heptachloroborane as a toxic component of toxaphene. However, there is little information available on the mode of action of toxaphene and its components. ATPase inhibition appears to be a possible mode of action of chlorinated hydrocarbon pesticides. In our research, we observed consistent but different patterns of inhibitory responses of ATPases to organochlorines. Chlordane and dicofol inhibited both Na^+-K^+ and Mg^{2+} ATPases in vertebrate and invertebrate tissues (KOCH 1969, CUTKOMP et al. 1971a). DDT and its related compounds showed high inhibition of mitochondrial Mg^{2+} ATPase in in vitro (CUTKOMP et al. 1971b, DESAIAH et al. In Press 1974) and in vivo (DESAIAH et al. 1974), studies in fish. Naphthalenes, like endrin and isodrin, showed no inhibition on Mg^{2+} ATPase and little effect on Na^+-K^+ ATPase in fish brain (unpublished data). A recent report showed that Mg^{2+} ATPase in a microsomal fraction of rainbow trout gill was more sensitive than Na^+-K^+ ATPase to several organochlorines, including toxaphene (DAVIS et al. 1972).

The present work was undertaken to determine the inhibition pattern of toxaphene in different tissues of catfish, Ictalurus punctatus. The reason for this study is that toxaphene is widely used in the Southeastern United States to control cotton pests. An understanding of the mode of action of this pesticides could help in developing conditions for its use to give optimum pest control with minimum environmental contamination.

Materials and Methods

The enzyme sources were brain, kidney, and gill tissues from pond-cultured channel catfish, Ictalurus punctatus. The tissues were dissected and homogenized in cold 0.32 M sucrose solution containing 1 mM EDTA and 10 mM Imidazole (pH 7.5). The homogenates were fractionated according to the procedure described by

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TABLE I

In vitro inhibition of catfish brain ATPases by toxaphene. Standard errors were calculated based on the mean values of separate enzyme determination of the number of homogenate preparations indicated in parentheses. Each homogenate was tested 2-3 times and the average taken. For reaction conditions, see Materials and Methods. Specific Activity = μmoles Pi Mg^{-1} Protein hr^{-1}.

Toxaphene (μM)	Na^+-K^+ ATPase		Oligomycin Sensitive Mg^{2+} ATPase		Oligomycin Insensitive Mg^{2+} ATPase	
	Specific Activity	% Inh.	Specific Activity	% Inh.	Specific Activity	% Inh.
none	14.68 ±1.48 (4)		5.23 ±0.57 (4)		11.21 ±0.75 (4)	
1.8	13.40 ±1.95 (2)	8.2	4.22 ±0.78 (2)	19.3	7.88 ±0.34 (2)	29.7*
3.6	10.82 ±1.02 (4)	25.9	3.95 ±0.28 (4)	24.5	6.45 ±0.42 (4)	42.5**
7.2	8.57 ±1.51 (4)	41.3*	2.82 ±0.71 (4)	46.1*	5.62 ±0.47 (4)	49.9***
14.5	7.60 ±0.90 (4)	48.0**	2.82 ±0.83 (4)	46.1	3.14 ±0.31 (4)	72.0***
29.0	7.30 ±0.31 (3)	50.0**	2.13 ±0.93 (3)	59.3*	2.83 ±0.47 (3)	74.8***

Statistically significant when determined by Student's t test, *P<.05; **P<.01; ***P<.001.

TABLE II

In vitro inhibition of catfish kidney ATPases by toxaphene. Standard errors were calculated based on the mean values of separate enzyme determinations of the number of homogenate preparations indicated in parentheses. Each homogenate was tested 2-3 times and the average taken. For reaction conditions, see Materials and Methods. Specific Activity = μmoles Pi mg^{-1} Protein hr^{-1}.

Toxaphene (μM)	Na^+-K^+ ATPase		Oligomycin Sensitive Mg^{2+} ATPase		Oligomycin Insensitive Mg^{2+} ATPase	
	Specific Activity	% Inh.	Specific Activity	% Inh.	Specific Activity	% Inh.
none	14.83 ±2.22 (4)		4.85 ±1.03 (4)		10.77 ±0.89 (4)	
1.8	16.08 ±0.46 (2)	+8.4[a]	7.26 ±0.48 (2)	+49.7	10.61 ±0.95 (2)	1.5
3.6	10.46 ±1.49 (4)	29.5	5.26 ±0.98 (4)	+8.5	9.05 ±0.59 (4)	16.0
7.2	10.14 ±1.53 (4)	31.6	5.00 ±0.75 (4)	+3.1	7.75 ±0.60 (4)	28.0*
14.5	9.80 ±1.65 (4)	33.9	3.74 ±0.19 (4)	22.9	6.87 ±0.44 (4)	36.2**
29.0	9.84 ±1.27 (4)	33.7	2.80 ±0.23 (4)	42.3	6.49 ±0.57 (4)	39.7**

[a](+)values indicate per cent stimulation of enzyme activity.
Statistically significant when determined by Student's t test, *P<.05; **P<.01.

KOCH (1969a). The 'B' fraction obtained at 13,000 g centrifugation was resuspended in sucrose solution and divided into small aliquots to contain 20-30 µg protein per 100 µl sample. These samples were quick frozen in liquid nitrogen and stored at -20°C until used for ATPase determination.

The ATPase activities were measured by a continuous procedure. A 3-ml reaction mixture contained 4.3 mM ATP, 135 mM Imidazole-Cl buffer (pH 7.5), 0.2 mM NADH, 0.5 mM phosphoenol pyruvate, 0.02 per cent bovine serum albumin (BSA), 5 mM Mg^{2+}, 100 mM Na^{+}, 20 mM K^{+} (These three as chlorides), approximately 9 units of pyruvate kinase, and 12 units of lactic dehydrogenase. One hundred µl of the homogenate fraction was used per 3-ml reaction mixture. Absorbance was measured at 340 nm using a Gilford 2400 automatic recording spectrophotometer with temperature controlled at 37°C. Protein concentration was determined by the method of Lowry et al. (1951). All chemicals (except the chlorides) used in the reaction mixture were obtained from Sigma Chemical Company. Ouabain at 1 mM concentration was used to differentiate Na^{+}-K^{+} ATPase from Mg^{2+} ATPase. Ouabain is a specific inhibitor of Na^{+}-K^{+} ATPase (McILWAIN 1963). Mg^{2+} ATPase was further delineated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive Mg^{2+} ATPases by adding 1 µl of oligomycin ethanol solution (0.5 mg/ml 95% ethanol) to the reaction mixture.

Toxaphene was obtained from Hercules Incorporated, Wilmington, Delaware. The 87-mM stock solution was prepared by dissolving toxaphene in an ethanol-acetone mixture. Further dilutions were made with ethanol only. Ethanol and acetone at the concentrations used in this study have no detectable effect on ATPase activity.

Results and Discussion

Oligomycin-insensitive Mg^{2+} ATPase in catfish brain showed a higher degree of sensitivity to toxaphene as compared to oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase (Table I). This observation is in contrast to DDT and its related compounds which showed a higher inhibition of mitochondrial Mg^{2+} ATPase with little or no effect on the oligomycin-insensitive Mg^{2+} ATPase in fish brain (CUTKOMP et al. 1971b, DESAIAH et al. 1974). However, toxaphene inhibited all three ATPases in fish brain to the extent of 50 per cent with a pronounced effect on the oligomycin-insensitive Mg^{2+} ATPase activity (Table I).

The results in Table II show that toxaphene has lesser inhibitory effect on kidney than on brain ATPases. Oligomycin-insensitive Mg^{2+} ATPase activity showed increased inhibition with increasing concentration of toxaphene (Table II). Kidney mitochondrial Mg^{2+} ATPase activity was stimulated at low concentrations and inhibited at higher concentrations of toxaphene. This type of response was also seen with some polychlorinated biphenyl compounds on sunfish kidney mitochondrial Mg^{2+} ATPase (DESAIAH et al. 1972, KOCH et al. 1972). Na^{+}-K^{+} ATPase activity in kidney gave about 30 per cent

TABLE III

In vitro inhibition of catfish gill ATPases by toxaphene. Standard errors were calculated based on the mean values of separate enzyme determination of the number of homogenate preparations indicated in parentheses. Each homogenate was tested 2-3 times and the average taken. For reaction conditions, see Materials and Methods. Specific Activity = μmoles Pi mg^{-1} Protein hr^{-1}.

Toxaphene (μM)	Na^+-K^+ ATPase		Oligomycin Sensitive Mg^{2+} ATPase		Oligomycin Insensitive Mg^{2+} ATPase	
	Specific Activity	% Inh.	Specific Activity	% Inh.	Specific Activity	% Inh.
none	9.94 ±0.98 (4)		5.52 ±0.41 (4)		15.48 ±1.18 (4)	
1.8	10.14 ±1.41 (3)	+2.0[a]	5.89 ±0.90 (3)	+6.7	11.20 ±1.01 (3)	27.6*
3.6	7.73 ±0.79 (4)	22.2	5.66 ±0.45 (4)	+2.5	10.23 ±1.15 (4)	33.9*
7.2	6.42 ±1.01 (4)	35.4*	4.47 ±0.46 (4)	9.0	8.08 ±0.73 (4)	47.8**
14.5	6.35 ±0.69 (4)	36.1*	3.26 ±0.29 (4)	40.9**	6.72 ±0.46 (4)	56.6***
29.0	6.22 ±0.99 (4)	37.4*	2.71 ±0.18 (4)	50.9***	6.11 ±0.35 (4)	60.5***

[a] (+) values indicate per cent stimulation of enzyme activity.
Statistically significant when determined by Student's t test, *P<.05; **P<.01; ***P<.001.

inhibition at all concentrations of toxaphene with an exception of one low dose (Table II). The reason for this type of response (maximum inhibition of 33%) is not understood, but we feel that it could possibly indicate the presence of more than one type of Na^+-K^+ ATPase activity.

The results in Table III show the inhibitory action of toxaphene on the ATPase activities from catfish gill tissue. The inhibition of the oligomycin-insensitive Mg^{2+} ATPase activity grom gill tissue (Table III) was similar to that obtained for brain (Table I), except that at the higher concentrations of toxaphene inhibition of the enzyme activity from brain was somewhat higher than from gill tissue. However, Na^+-K^+ and mitochondrial Mg^{2+} ATPases in gill tissue (Table III) showed a similarity in inhibition with that of kidney tissue (Table II). Both of these latter enzymes showed slight (but not significant) stimulation by toxaphene at low concentrations and inhibition at higher concentrations.

All three ATPases in the three tissues of catfish tested showed inhibition by toxaphene. The responses of ATPases to toxaphene varied from tissue to tissue and within the same tissue. However, greatest inhibition occurred for oligomycin-insensitive Mg^{2+} ATPase in brain and gill tissues. This differential sensitivity of ATPases to toxaphene could be due to the fact that toxaphene is a mixture of 175 components and each toxic component may be exerting different effects on the various ATPase activities. The most desirable approach to elucidate the mode of action of toxaphene would be by studying the inhibitory patterns of individual toxic components of toxaphene. We are planning to initiate such a study in our laboratory.

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