INHIBITION OF FISH BRAIN ATPases BY ALDRIN-TRANSDIOL, ALDRIN, DIELDRIN AND PHOTODIELDRIN

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SUMMARY

The sensitivity of catfish, Ictalurus punctatus, brain ATPase activities to cyclodiene compounds was investigated. The ATPase system showed differences in sensitivity to aldrin, dieldrin and photodieldrin. However, aldrin-transdiol (a more terminal metabolite of dieldrin and reported as a more potent neurotoxin than dieldrin) had no effect on any ATPase activity from fish brain homogenates. Mitochondrial Mg^{2+} ATPase was the most sensitive ATPase to the cyclodiene compounds tested. The possibility that the neurotoxic effects of these compounds is a secondary response resulting from mitochondrial Mg^{2+} ATPase inhibition is discussed.

Aldrin-transdiol, a metabolite of dieldrin, has been reported to be more potent in exerting a neurotoxic action than dieldrin (1-3). However, Shroeder and Shankland were unable to detect any neurotoxic effect of aldrin-transdiol on metathoracic ganglion of cockroach (4). In view of the above different findings on similar preparations we decided to examine the effects of aldrin-transdiol along with aldrin, dieldrin and photodieldrin on the ATPase system, because we had previously observed variations in inhibitory effects on ATPase activities by hydroxylated chlorinated hydrocarbon pesticides (5) (unpublished data).

The epoxidation of aldrin to dieldrin (6-8) and the metabolism of dieldrin to several more polar metabolites in several organisms have been extensively investigated (6, 7-12). Aldrin-transdiol has been identified as a dieldrin metabolite in insects and mammals (13-17). Recently it was reported that aldrin-transdiol was more rapid and potent than dieldrin in exerting effects on: a. synaptic transmission across the metathoracic

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ganglion of the cockroach (1), b. the frog sciatic nerve - sartorius muscle preparation (2), and c. the squid giant axons (3). However, more recently, Shroeder and Shankland were unable to produce the neurotoxic responses by aldrin-transdiol in preparations of the metathoracic ganglion of the cockroach (4). Based on their experimental evidence, these authors (4) questioned the theory that aldrin-transdiol is the active metabolite of of dieldrin.

The present investigation was designed to determine the effects of aldrin-transdiol on the ATPase system and to see if this approach could add useful information for understanding the mode of action of dieldrin and aldrin. In our earlier studies, we observed that the ATPase system was sensitive to organochlorine pesticides both in vitro (5,18) and in vivo (20). It was also found that different types of organochlorines had different effects on the three apparently different ATPase activities. For example, DDT was more effective on the mitochondrial Mg^{2+} ATPase activity (5,18,19), whereas chlordane was equally effective on Na^{+} - K^{+} and Mg^{2+} ATPase activities (18), and some polychlorinated biphenyls and toxaphene showed greater inhibition on the oligomycin-insensitive Mg^{2+} ATPase activity (21, 22). Because of these differences of sensitivity, we thought that the study of this enzyme system could provide a possible method for further elucidating the action of aldrin-transdiol along with aldrin, dieldrin and photodieldrin.

Brain tissue from channel catfish, Ictalurus punctatus, was dissected, homogenized and fractionated according to the procedure described by Koch (23). The 13,000 x g sediment fraction containing the mitochondria plus nerve ending particules was utilized in this study. The sediment was resuspended in a 0.32 M sucrose solution to contain 20 - 25 µg protein per 50 µl sample. These samples were then quick frozen in liquid nitrogen and stored at $-20^{\circ}C$. The ATPase activities were determined by a continuous procedure described earlier (24). Protein concentration of the samples was determined by the method of Lowry et al. (25). The description of the assay and the reaction conditions is given in Table 1.

Table 1. Sensitivity of Catfish Brain ATPases to Cyclodiene Insecticides

Compound	(μM)	Specific Activity ± S.E.*		
		Na^+-K^+ ATPase	Mg^{2+} ATPase	
			Oligomycin	
			Sensitive	Insensitive
None		18.0 ± 0.4	11.7 ± 1.1	15.3 ± 0.4
Aldrin				
	5.0	13.1 ± 1.0	7.4 ± 1.0	4.8 ± 0.5
	20.0	10.5 ± 0.6	4.0 ± 0.03	3.1 ± 0.5
Dieldrin				
	1.25	18.1 ± 1.8	7.5 ± 0.6	11.7 ± 0.9
	5.0	16.3 ± 1.3	5.2 ± 0.3	8.3 ± 0.1
	20.0	29.6 ± 3.1	6.1 ± 0.03	7.9 ± 0.7
Photodieldrin				
	5.0	16.8 ± 1.8	9.0 ± 0.6	13.2 ± 0.2
	20.0	15.2 ± 1.1	4.6 ± 0.06	10.6 ± 0.4
Aldrin-transdiol				
	5.0	18.4 ± 1.1	12.0 ± 1.6	14.9 ± 0.1
	20.0	19.0 ± 0.8	11.4 ± 1.3	14.8 ± 0.1

A 3 ml. reaction mixture contained compounds at the following concentrations: 4.5 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazole buffer (pH 7.5),0.19 mM NADH, 0.5 mM PEP, 0.02% BSA, approximately 9 units pyruvate kinase and 12 units lactic dehydrogenase, and 50 μl. homogenate fraction (20-25 μg protein). Absorbance changes were measured at 340 nm for 15 min using a Gilford Recording spectrophotometer with temperature controlled at 37°C in reaction mixture. When used, ouabain was present at 1.0 mM. Total ATPase activity was determined in the presence of Na^+, K^+ and Mg^{2+}. Mg^{2+} ATPase activity was measured using same mixture plus ouabain. Na^+-K^+ ATPase activity is total activity minus Mg^{2+} ATPase activity. Oligomycin (2 μg/ml reaction mixture)was used to delineate oligomycin-sensitive (mitochondrial) and insensitive Mg^{2+} ATPases. Ethanol was used as solvent for the insecticides and 1 μl of each solution was added to the enzyme reaction mixture by slowly releasing the solution from a Hamilton microsyringe into a rapidly stirred reaction mixture.

* Standard errors were calculated based on the mean values of three separate homogenate preparations. Specific activity is expressed as μmoles Pi mg^{-1} protein hr^{-1}.

A highly purified sample of photodieldrin (26) was kindly provided by Drs. Khan and Reddy.* Photodieldrin and the other compounds used were dissolved in ethanol and 1 µl of the required strength of the insecticide solutions was slowly added to the reaction mixture from a Hamilton microsyringe with rapid stirring.

The results obtained are summarized in Table 1. Among the compounds tested, aldrin showed the highest inhibition of the three ATPase activities in the fish brain fraction. However, the aldrin inhibition was greater on Mg^{2+} ATPase than on the Na^{+} - K^{+} ATPase activities. Photodieldrin, a photoconversion product of dieldrin, shown to be more toxic than dieldrin (27,29), also inhibited all three ATPases with a greater effect on Mg^{2+} ATPases than on Na^{+}- K^{+} ATPase activity (Table 1). Dieldrin at 5 µM concentration was more effective than photodieldrin on the oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase, but, at 20 µM the reverse was true, i.e., photodieldrin was more effective than dieldrin (Table 1). Also, the results in Table 1 and Figure 1 show that dieldrin at 20 µM stimulated the Na^{+}- K^{+} ATPase activity, while photodieldrin inhibited this enzyme. The stimulation produced by dieldrin was time dependent as shown in Figure 1. The reason for this effect is not apparent at this time. We have not observed this type of stimulation with any of the other organochlorine pesticides tested.

Aldrin-transdiol, a metabolite of dieldrin, did not inhibit any of the ATPase activities in the catfish brain homogenate fraction used in these studies. Previously, we have observed that the organochlorines with hydroxyl groups produced a greater inhibition of the ATPase activity. For example, Dicofol^{R} was more effective than DDT on blue gill fish brain ATPase system (5). Because of this latter finding, we assumed that aldrin-transdiol would be more effective than aldrin and dieldrin due to the presence of the two hydroxyl groups in the former. However, the results obtained (Table 1) show

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that aldrin-transdiol had no effect on the ATPase activities from catfish brain.

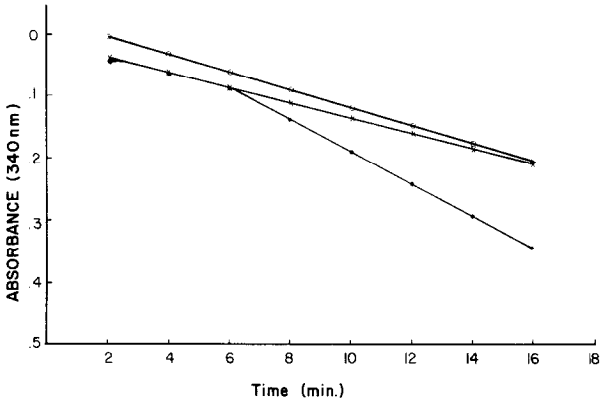

Fig. 1. Effect of dieldrin and photodieldrin (20 μM) on $Na^+ - K^+$ ATPase activity in catfish brain homogenate (0-0) control, (●-●) dieldrin and (x--x) photodieldrin. For reaction conditions see Table 1.

Although the *in vitro* results of ATPase inhibition cannot be directly correlated to the known toxicity values of these insecticides, it is of interest to relate the reported toxicology data of these insecticides with our ATPase inhibition results. Aldrin-transdiol has been reported to be far less toxic to mice than aldrin and dieldrin (LD_{50}'s -1250, 95 and 70-100 mg/Kg respectively) when administered orally (17,30,31). Shroeder and Shankland (4) found that aldrin-transdiol was non-toxic to cockroaches over a period of 1 week at an internal concentration of 1×10^{-3}M, whereas dieldrin at similar concentration gave 80% mortality in ten hours. Photodieldrin was more toxic than dieldrin to several aquatic and terrestrial animals (27-29). The results in Table 1 show that aldrin-transdiol did not effect any of the ATPase activities at the levels tested, while the other three compounds strongly inhibited the oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase activity.

If we relate the inhibition of the mitochondrial Mg^{2+} ATPase to the toxicity of the organochlorine pesticides as in the case of the DDT group (19),

then the neurotoxic action of some of these tested compounds as reported by others (1,3,32) may be due to a secondary response. It may be possible that dieldrin and certain of its metabolites collectively are responsible for the observed toxic action of this pesticide in vivo. On the other hand, a particular metabolite of dieldrin could exert the major toxic action, since it is known that a latent period of several hours is required before toxic symptoms are observed (32). For example, it has been reported (33) that Klein's metabolite, ketodieldrin, [a keto derivative of photodieldrin (28, 34)] was more toxic to houseflies than either aldrin or dieldrin. Further studies on metabolites of dieldrin, both in vitro and in vivo, may produce definitive results on the mode of action of these pesticides.

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