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Inhibition of ATPases Activity in *Torpedo* Fish by DDT, Dicofol, and Chlorobenzilate Pesticides

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Abstract. The specific activities of the mitochondrial Na^+, K^+ - and Mg^{2+} - ATPases, from different organs of *Torpedo* fish, have been measured. The enzyme Na^+, K^+ - ATPase, from electric organ and brain tissues of the *Torpedo* fish, showed greater activity than that, extracted from the muscle and liver tissues. The activities of Mg^{2+} - ATPase, obtained from the muscle and liver tissues were found to be more active than that, obtained from the tissues of electric organ and brain.

The measurements of in vitro inhibition of both enzymes by certain neurotoxic pesticides: DDT, dicofol and chlorobenzilate, as well as, by the specific inhibitor, ouabain, were assessed. The results showed that, DDT caused strong inhibition on the muscle and liver Mg^{2+} - ATPases, I_{50} values were 8 and 10 μM respectively. However, dicofol showed strong inhibitory effects on the electric organ or brain Na^+, K^+ - ATPases activities, with I_{50} values equal to 6 and 14 μM respectively. Chlorobenzilate showed very slight inhibitory effects on the activities of both enzymes. Ouabain showed very strong inhibition of the electric organ Na^+, K^+ - ATPase activity with an I_{50} value equal to 4 μM.

Introduction

The mechanism of toxic action of DDT and its analogs are not clearly understood, even though, it has been accepted that, DDT acts directly on the nervous system, causing distruption of normal ion permeablities in the nerve membrane, that are implicit in the generation and conduction of nerve impulses [1]. It is also well-known that DDT inhibits several Ca^{2+} - independent ATPases, including ouabain - sensitive Na^+, K^+, ATPase of axon membrane of the lobster (*Homarus americanus*) [2], and mitochondrial Mg^{2+} - ATPase [3]. However, recent reports describe DDT as a potent inhibitor of a Ca^{2+} - dependent ATPase of the lobster peripheral axons [4,5]. In vivo tests, carried out on houseflies by Patil *et al.* [6] showed that, DDT was a strong inhibitor of oligomycim - sensitive (OS) Mg^{2+} - ATPase, and a very weak inhibitor of Na^+, K^+ - ATPase.

Reports showed that, chorinated hydrocarbons, especially DDT analogs, were dissimilar to DDT in their mode of action [7,8]. An increased inhibition of Na^+, K^+ - ATPase, by increased concentration of dicofol and kepone on bluegill brain Na^+, K^+ - ATPase was observed [9,10]. However, a number of other chlorinated hydrocarbons, which inhibited Na^+, K^+ - ATPase, did not show such progressive response.

The present research was carried out to investigate the sensitivity of Na^+, K^+ - ATPase and Mg^{2+} - ATPase, extracted from different tissues (electric organ, brain, nerve cord, muscles and liver) of the *Torpedo oscellata* fish to DDT and its carbinol analogs: dicofol and chlorobenzilate.

Materials and Methods

Tissues from *Torpedo* fish were dissected and homogenized in a solution of 0.32 M sucrose, 1mM EDTA and 40 mM Tris buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial ATPase was prepared according to the method reported by Koch [11], by differential centrifugation of the homogenate at 8 000 Xg for 10 min. The supernatant was then centrifuged at 2 0000 Xg for 30 min. The formed pellets were then suspended in the buffer and stored at -40°C for use.

The ATPase activity measurements were done according to the method reported by Koch [11] with slight modification, using tris- HCl buffer instead of imidiazol buffer. The method was based on the spectrophotometric determination of the inorganic phosphate (P_i) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity was measured in a total volume of 1 ml. The mitochondrial preparation was mixed with a reaction mixture (700 μl) containing 100 mM Na^+, 20 mM K^+, 5 mM Mg^{2+} chlorides, 40 mM Tris - HCl buffer (pH 7.4) and 5 mM ATP. The volume was completed to 850 μl with the buffer. The mixture was incubated for 15 min. in a shaking water bath at 37°C. The reaction was stopped by adding 150 μl trichloroacetic acid (TCA, 30%). Hydrolyzed P_i was determined according to the method, described by Taussky and Shorr [12]. The activity of Mg^{2+} - ATPase was measured after the addition of 1 mM ouabain, a specific inhibitor for Na^+, K^+ - ATPase [11], whereas the activity of Na^+, K^+ - ATPase was calculated as the difference between the total ATPase and Mg^{2+} - ATPase activities.

From appropriate concentrations of the tested insecticides (DDT, dicofol and chlorobenzilate), 10 μl (in ethanol) was incubated with the enzyme mixture for 30 min, before the addition of the substrate (ATP, 5mM), then the reaction mixture was reincubated for another 15 min. at 37°C.

Inhibition of the enzyme activity was calculated as percentage of inhibition (%I), from the values of the absorbance at λ_{max} 740 nm. as follows:

$$\% I = 100 (A - A_i)/A$$

where A = is the absorbance in the control (without insecticide)

A_i = is the absorbance in tested samples (with insecticide)

The enzyme protein determination was carried out according to the method of Lowery *et al.* [13].

Results and Discussion

Specific activities of ATPases from different tissues

Results recorded in Table 1 for specific activities of the ATPases, derived from different tissues of *Torpedo* fish, showed the following points:

1- Maximum value for specific activity of Na^+, K^+ - ATPase was found in electric organ, whereas that of Mg^{2+} - ATPase was measured in muscle preparation.

2- Values of Na^+ , K^+ - and Mg^{2+} - ATPases activities in brain, liver and muscle preparations of the *Torpedo* fish, were comparable to those of bluegill fish [10].

3- Total activities of ATPase (both enzymes) were greatest (91.6±1.7) in electric organ preparation, and least in the liver (34.4±2.1). Total ATPase activities were modest in the brain, nerve cord and the muscle preparations (the values are 58.6±1.8, 52.8±1.5 and 60.1±1.1 respectively).

4- Relatively low ATPase activities of muscle or liver Na^+, K^+ - ATPase, as well as those of electric organ or brain Mg^{2+} - ATPase render them less sensitive to different inhibitors. For the same reason, electric organ or brain Na^+, K^+ - ATPase as well as muscle or liver Mg^{2+} - ATPase, were commonly used in the in vitro ATPase inhibition measurements.

Inhibition studies

Ouabain, as presented in Fig. 1, showed a value of 4×10^{-6} M for the half maximal inhibition (I_{30}), for the *Torpedo* electric organ Na^+, K^+ - ATPase and a concentrate of 1×10^{-3}M for the 95 - 98% inhibition of the enzyme activity. These results are in agreement with those of several investigators [14-16]. However pI_{50} values of ouabain on Na^+, K^+ - ATPase may be higher than those mentioned, the value of 3.9 was reported for rat liver [17] and 5.75 for eye preparation of blowfly [18]. This would appear to indicate that, compared to these tissues, the Na^+, K^+ - ATPase of electric organ preparations from *Torpedo* fish is in the same level of sensitivity to ouabain, with those from locust.

Table 1. Specific activities of ATPases from different tissues of *Torpedo* fish

Tissue	Specific activity (μ mole P_i/mg Protein/hr.)±SE		
	Total ATPase	Na^+, K^+-ATPase	Mg^{2+}-ATPase
Electric organ	91.6±1.7	84.5±1.2	7.1±2.2
Brain	58.6±1.8	49.3±2.1	9.3±1.7
Nerve cord	52.8±1.5	38.6±1.6	14.2±2.1
Muscle	60.1±1.1	6.5±1.3	53.6±1.6
Liver	34.4±2.1	3.1±1.1	31.3±2.3

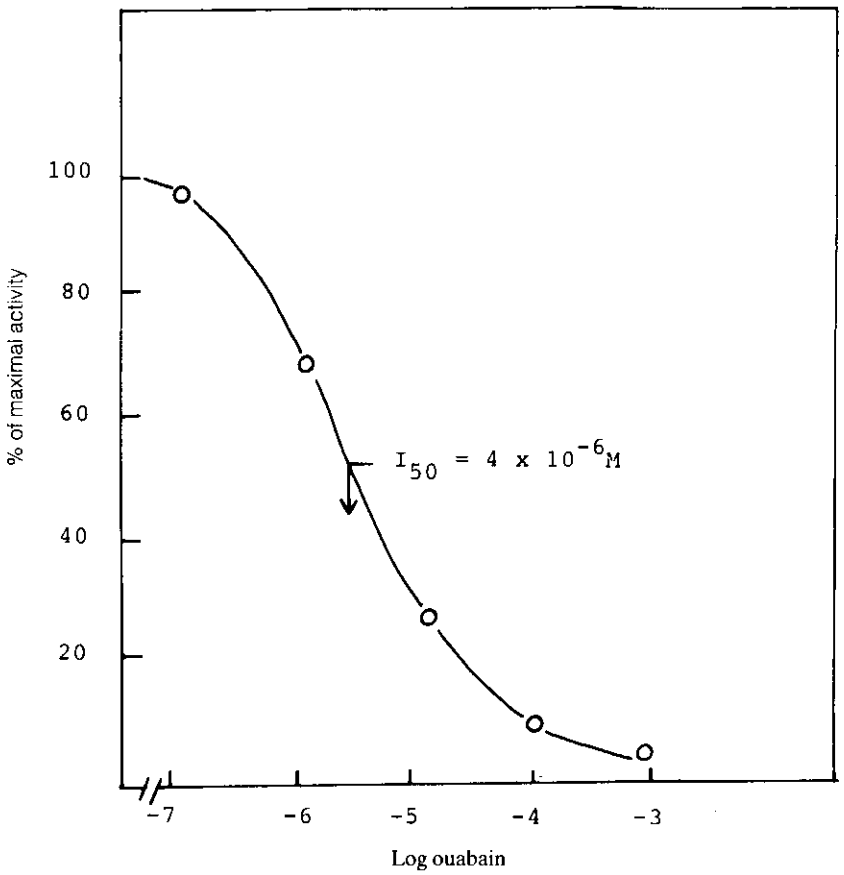

Fig. 1. The effect of ouabain on the activity of Torepdo electric organ Na^+, K^+-ATPase

From the view point of the chemical structure, the three tested chlorinated hydrocarbons: DDT, dicofol and chlorobenzilate are related to each other. Dicofol is a carbinol analog of DDT, whereas chlorobenzilate is a methyl-carboxylate analog of dicofol. Hence, dicofol and chlorobenzilate could be considered as carbinol analogs of DDT.

Previous findings indicated that, chlorinated hydrocarbons, especially DDT analogs were dissimilar to DDT in their biological behaviour or mechanisms of toxicity [8]. It has also been reported by Sawicki [7] that knock-down resistant [kdr) and super-kdr strains of house fly to DDT did not extend to the carbinol analogs, such as dicofol.

Results presented in Table 2, show that DDT is a strong inhibitor to Mg^{2+} - ATPase (in muscle and liver preparations), showing values for I_{50} equal to 8 and 10 μM respectively. Its inhibitory effects to Na^+, K^+ - ATPase from electric organ or brain were five times less than those of Mg^{2+} - ATPases. The corresponding I_{50} values were 40 and 50 μM respectively. Such results agree with those of Patil *et al.*[6] who reported that, in vivo, DDT was a strong inhibitor of Mg^{2+} - ATPase (oligomycin sensitive enzyme) and a very weak inhibitor of Na^+, K^+ - ATPase. Desaiah *et al.* [19] observed greater inhibition of Mg^{2+} - ATPase of fish brain, both in vitro and in vivo by DDT. It has been reported by Doherty and Matsumura [2] and Cheng and Cutkomp [3] that DDT inhibited several Ca^{2+} - independent ATPases including ouabain sensitive Na^+, K^+ - ATPase of axon membrane and mitochondrial Mg^{2+} - ATPase. However, Morshedy *et al.* [20] observed a weak correlation between the DDT - toxicity to *Heliothis zea* larvae, and its inhibitory potential of ATPase. They reported that, 3rd instar larval ATPase was 2.5 times less sensitive to DDT, than the 5th instar while DDT was 5-times more toxic to the 3rd instar than the 5th instar.

DDT at its maximum concentration (100 μM) caused 62 and 58% inhibition in electric organ and brain Na^+, K^+ - ATPases respectively (Table 2). Such weak response, has been previously reported for a number of chlorinated hydrocarbons, that showed a maximum inhibition of 30 - 50% in Na^+ , K^+ - ATPase [9,10,19].

Contrary to DDT, dicofol proved to be a powerful inhibitor to the electric organ or brain Na^+, K^+ - ATPase and to a lesser extent to the muscle or liver Mg^{2+} - ATPase. For the latter enzyme, dicofol was less potent as an inhibitor, than DDT (Table 2). It seems that, electric organ Na^+, K^+ - ATPase was more sensitive to be inhibited by dicofol than corresponding brain - enzyme, whereas Mg^{2+} - ATPases from either muscles or liver, equally responded to the dicofol inhibition; and the corresponding brain - equally responded to the dicofol inhibition; and the corresponding I_{50} values were 26 or 27 μM.

Table 2. Percent inhibition of ATPase from different tissues of *Terpedo* fish by DDT and some related compounds.

Enzyme & source	Concentrations (μM)					I_{50} (μM)
	1	5	10	50	100	
			DDT			
Na^+, K^+-ATPase						
Electric organ	16	26	38	51	62	40
Brain	12	22	31	46	58	50
Mg^{2+}- ATPase						
Muscle	26	43	52	71	89	8
Liver	22	36	49	68	84	10
			Dicofol			
Na^+, K^+-ATPase						
Electric organ	26	47	60	74	86	6
Brain	22	35	52	60	72	14
Mg^{2+}-ATPase						
Muscle	12	22	34	61	72	26
Liver	16	23	32	58	71	27
			Chlorobenzilate			
Na^+, K^+-ATPase						
Electric organ	-	8	22	40	52	85
Brain	-	-	12	31	44	> 100
mg^{2+}-ATPase						
Muscle	6	16	24	46	58	60
Liver	5	12	21	38	51	90

Chlorobenzilate behaved differently, it showed slight inhibitory effect of the Na^+, K^+ - ATPases and Mg^{2+} - ATPases as well. Its effects on Mg^{2+} - ATPases, were slightly higher than those on Na^+, K^+ - ATPases.

The present results, showed that, DDT and its carbinol analogs: dicofol and chlorobenzilate were dissimilar in their inhibitory effects on the Na^+, K^+ - and Mg^{2+} - ATPases of the *Torpedo* fish, such behaviour was also observed in their mechanism of toxicity as previously stated by Beeman [8].

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تثبيط نشاط الأنزيمات المحللة للأدينوزين ثلاثي الفوسفات في سمك التوربيدو بواسطة مبيدات الـ د. د. ت. والديكوفول والكلوروبنزليت

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ملخص البحث. تم تقدير النشاط النوعي لإنزيمي أدينوزين ثلاثي الفوسفات (صوديوم وبوتاسيوم) وأدينوزين ثلاثي الفوسفات (ماغنسيوم) المستخلصين من ميتوكوندريا الأعضاء المختلفة من سمك التوربيدو. وجد أن إنزيم أدينوزين ثلاثي الفوسفات (صوديوم وبوتاسيوم) المستخلص من العضو المكهرب ونسيج المخ ذات نشاط عالٍ عن تلك المستخلصة من أنسجة العضلات والكبد. وأن نشاط إنزيم أدينوزين ثلاثي الفوسفات (ماغنسيوم) المستخلص من العضلات والكبد أعلى من المستخلص من العضو المكهرب والمخ.

تم تقدير القياسات التثبيطية لكل من الإنزيمين بواسطة مبيدات الـ د. د. ت. والديكوفول والكلوروبنزليت وأيضًا بواسطة المثبط المتخصص الأوابين. وجد أن الـ د. د. ت. أظهر قدرة تثبيطية على نشاط إنزيم أدينوزين ثلاثي الفوسفات (ماغنسيوم) المستخلص من العضلات والكبد وكان التركيز المثبط لـ ٥٠٪ من نشاط الإنزيم هو ٨، ١٠ ميكرومولر على التوالي. بينما أظهر الديكوفول قدرة تثبيطية عالية على نشاط إنزيم أدينوزين ثلاثي الفوسفات (صوديوم وبوتاسيوم) المستخلص من العضو المكهرب والمخ وكان التركيز المثبط لـ ٥٠٪ من النشاط هو ٦، ١٤ ميكرومولر على التوالي. بينما أظهر الكلوروبنزليت تأثيراً ضعيفًا على كل من الإنزيمين.

أظهر الأوابين قوة تثبيطية عالية على نشاط إنزيم أدينوزين ثلاثي الفوسفات (صوديوم وبوتاسيوم) وكان التركيز المثبط لـ ٥٠٪ من نشاط الإنزيم ٤ ميكرومولر.