Bull. Environm. Contam. Toxicol. 20,505-512 (1978)

Comparative Study of Respiratory Chain Inhibition by DDT and DDE in Mammalian and Plant Mitochondria

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The problem of DDT[1] as an environmental component that affects living organisms (for review see BYCZKOWSKI 1974 and 1976) will be acctual for a long time - at least some 30 years. A few recent studies by BYCZKOWSKI (1976a) have showed some data suggesting that toxicity of DDT in mammals may be related to disruption of energy conservation process and inhibition of electron transfer in mitochondria.

It has been also shown that DDT and DDE affected the electron-transfer chain in chloroplasts (for review see BYCZKOWSKI 1974). However, effect of these compounds on plant mitochondria has been not established since now. The persistance of DDT in soil is well known and, therefore it was of interest to investigate the effect of this pesticide on mitochondria from germinating cereals seedlings.

The aim of present paper is to compare effects of DDT as well as DDE on rat liver and brain mitochondria with those on wheat and rye seedlings mitochondria.

MATERIALS AND METHODS

Rat liver and brain mitochondria.

Rat liver mitochondria were prepared by the method of WEINBACH (1961) essentially as described earlier (BYCZKOWSKI 1973).

Rat brain mitochondria were prepared by the method described by CHAPPELL and HANSFORD (1972). The homogenization medium used for preparation of rat liver and brain mitochondria contained: 0.25 M sucrose, 10 mM tris-HCl /pH 7.3/ and 1 mM EGTA buffered with KOH /pH 7.3/.

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[1]Abbreviations used: DDT - 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane; DDE - 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene.

0007-4861/78/0020-0505 $01.60

Respiration rates were measured with a Clark oxygen electrode at 25°C. The reaction mixture /final volume of 3.0 ml/ contained: 15 mM KCl, 50 mM tris-HCl /pH 7.3/, 5 mM $MgSO_4$, 5 mM potassium phosphate and 0.2 mM EGTA-KOH . Succinate 10 mM /+ glutamate 10 mM/ or malate 10 mM /+ glutamate 10 mM/ were used as substrates.

Plant mitochondria.

Plant mitochondria were prepared from dark-grown 2 - 2.5 days old shoots of wheat /Triticum aestivum L. var. Grana/ and rye /Secale coreale L. var. Dańkowskie Złote/. The seeds were germinated and seedlings grew on moist filter paper in Petri dishes at 21°C.

The mitochondria were isolated essentially as described by POMEROY (1974) with slight modifications in grinding and washing media. The grinding media contained: 0.5 M mannitol /for rye shoots/ or 0.4 M mannitol /for wheat shoots/, 1 mM EDTA, 0.1% (w/v) bovine serum albumin /BSA/ and 67 mM potassium phosphate buffer at pH 7.2. The mitochondrial pellet was resuspended in 20 ml of washing medium contained: 0.5 M mannitol /for rye/ or 0.4 M mannitol /for wheat/, 1 mM EGTA, 0.1% BSA and 67 mM potassium phosphate /pH 7.2/. The final mitochondrial preparation was resuspended in 1.0 ml of medium contained: 0.5 M mannitol /or 0.4 M mannitol/, 1 mM EGTA, 0.1% BSA and 10 mM tris-HCl /pH 7.2/.

Mitochondrial preparations /in amounts corresponding to 0.6 - 0.8 mg protein/ were added to the electrode vessel, and allowed to equilibrate for 2 min. The reaction mixture /final volume of 3.0 ml / contained: 0.5 M mannitol /for rye/ or 0.4 M mannitol /for wheat/ 10 mM KCl, 5 mM $MgSO_4$, 10 mM KH_2PO_4, 10 mM tris-HCl /pH 7.2/, 0.2 mM EGTA and 0.1% BSA. Substrate solutions /pH 7.2/ were added to give a final concentration of 10 mM malate /+ 10 mM glutamate/ or 10 mM succinate /+ 10 mM glutamate/.

Mitochondrial protein concentration was estimated in BSA-free suspensions by the biuret method (LAYNE 1957) using serum albumin as a standard.

Ratios ADP:O and R.C.R. were determinated by the method of ESTABROOK (1967) after addition of a known amount of ADP to induce the state 3 respiration.

Reagents.

Substrates, nucleotides etc. used were obtained either from Koch-Light Ltd. or International Enzymes Ltd. Carbonyl cyanide-m-chlorophenyl hydrazone /CCCP/ was obtained from Całbiochem. Ethylene glycol-bis(beta-aminoethyl ether) N,N'-tetraacetic acid /EGTA/, oligomycin, antimycin A and rotenone were obtained from Sigma Chem. Co. Pure DDT and its derivatives were generously donated by Dr J.Krechniak (Dept.Chemical

Toxicology, Gdańsk). All other reagents were of analytical grade and water redestilled from quartz was used throughout the work.

RESULTS

From comparison of pesticide concentrations that caused 50% of inhibition /I_{50}/ of state 3 respiration with succinate and malate by mitochondria from the four different sources (TABLE 1) it may be seen that rat liver mitochondria were more sensitive to DDT and DDE than the other.

TABLE 1

DDT and DDE - induced inhibition of state 3 respiration of mitochondria from different sources

source of mitochondria	I_{50} values (nmoles of pesticide / mg of protein)			
	DDT		DDE	
	succinate (+ glut.)	malate (+ glut.)	succinate (+ glut.)	malate (+glut.)
rat liver	187	120	187	133
rat brain	400	260	360	230
rye seedlings	375	260	360	300
wheat seedlings	370	265	365	315

However, it may be also seen that in every case malate /+ glutamate/ oxidation was more sensitive to inhibition than succinate /+ glutamate/ oxidation. It is worth to notice that rye seedlings, wheat seedlings and rat brain mitochondria were equally sensitive to both DDT and DDE.

Effects of DDT on oxidative phosphorylation /characterized by respiratory control ratio - R.C.R. and by ADP:O ratio/ in rye and wheat seedlings mitochondria as well as rat liver and brain mitochondria are presented in TABLE 2 and 3 respectively. It may be seen that in rye and wheat seedlings mitochondria DDT / 50 nmoles per mg of protein/ affected rather respiratory control ratio than oxidative phosphorylation efficiency /measured as ADP:O ratio/. In contrast to in rat liver and brain mitochondria the same dose of DDT decreased the R.C.R. parallel to ADP:O ratio (TABLE 2 and 3). At this low concentration DDT had not inhibitory effect on state 3 respiration, whereas even slight stimulation was noticed at the state 4.

TABLE 2

The effect of DDT on oxidative phosphorylation in rye and wheat seedlings mitochondria

substrate		rye mitochondria		wheat mitochondria	
		R.C.R.	ADP:O	R.C.R.	ADP:O
malate (+ glut.)	control	3.6	2.1	5.2	2.4
	DDT[x]	2.6	1.8	4.2	2.2
succinate (+ glut.)	control	2.5	1.2	2.2	1.9
	DDT[x]	1.5	1.1	1.5	1.3

[x]DDT at concentration of 50 nmoles per mg of protein was used. Values are representative results of experiments repeated at least three times. For experimental details see methods.

TABLE 3

The effect of DDT on oxidative phosphorylation in rat liver and brain mitochondria

substrate		liver mitochondria		brain mitochondria	
		R.C.R.	ADP:O	R.C.R.	ADP:O
malate (+ glut.)	control	6.1	2.9	3.5	1.7
	DDT[x]	5.0	1.1	2.3	0.9
succinate (+ glut.)	control	4.7	1.9	4.7	1.0
	DDT[x]	3.5	1.0	3.3	b.d

[x]DDT at concentration of 50 nmoles per mg of protein was used. Values are representative results of experiments repeated at least three times.
b.d.- result below detectable value. For experimental details see methods.

Such an effect of low doses of DDT and DDE was repeatedly noticed using mitochondria from all four sources employed.

DISCUSSION

In previous paper BYCZKOWSKI (1973) showed that DDT and its metabolites acted as inhibitors of respiratory chain /first of all at the site between NADH and CoQ/ as well as uncouplers of oxidative phosphorylation in rat liver mitochondria. Moreover, from the structure-activity relationship study it was concluded that with increasing lipophility of bis(p-chlorophenyl) ethane derivatives, increased also their ability to inhibition of electron transfer chain (BYCZKOWSKI 1976 b). On the other hand, from comparison of doses of DDT that caused uncoupling of oxidative phosphorylation with those resulted in respiratory chain suppression it appeared that oxidative phosphorylation system is more sensitive to DDT than respiratory chain in rat liver mitochondria (BYCZKOWSKI 1974). Similar points of action of DDT were shown in brain and liver mitochondria of rats treated with single, sublethal doses of p,p'-DDT (BYCZKOWSKI 1976a). Results presented in this paper supported those findings.

Plant mitochondria, however, have different sensitivity to oxidative inhibitors and uncouplers of oxidative phosphorylation than rat liver mitochondria (IKUMA and BONNER 1967 and 1967 a). Effects of classical inhibitors and uncouplers on wheat mitochondria were presented by SRIVASTAVA and SARKISSIAN (1970) and by POMEROY (1975). Indeed, in our experiments (not shown here) as high concentrations as 600 nmoles of 2,4-DNP per mg of protein were effective on wheat or rye mitochondria, whereas 10 nmoles per mg was enough in rat liver or brain preparations. Similarly at least 40-times higher concentrations of rotenone per mg of protein were wanted for inhibition of NAD--dependent substrate oxidation in wheat or rye mitochondria when compared with rat liver or brain mitochondria.

In contrast to that DDT and DDE were equally effective inhibitors of respiratory chain in rat brain and rye as well as wheat seedlings mitochondria (TABLE 1). Rat liver mitochondria, however, were some two-fold more sensitive. Similar dose-dependent inhibition in rye seedlings mitochondria to that in brain mitochondria suggested the same or at least very similar mode of action (not shown here). The point of action of DDT and DDE within the respiratory chain of rat brain, wheat seedlings and rye seed-

lings mitochondria appeared to be, first of all, between NADH and CoQ - similarly to that postulated in rat liver mitochondria (BYCZKOWSKI 1973). In higher concentrations, however, they are able to inhibit also succinate oxidation (see also review by BYCZKOWSKI 1974).

On the other hand, oxidative phosphorylation processes in wheat and rye seedlings mitochondria appeared to be more resistant to DDT-induced uncoupling than that in rat liver and brain mitochondria (TABLE 2 and 3). Addition of DDT or DDE to wheat and rye seedlings mitochondria (TABLE 2) resulted in reduction of respiratory control ratio (R.C.R.) rather than oxidative phosphorylation efficiency (measured as ADP:O), whereas in rat liver and brain mitochondria both R.C.R. and ADP:O were reduced markedly (TABLE 3).

This apparent contradiction may be explained by fact that respiratory control ratio is an indicator of mitochondrial membrane integrity rather than oxidative phosphorylation efficiency. Since, it was demonstrated that DDT and DDE caused membrane damage in rat liver mitochondria (BYCZKOWSKI 1977) it is logically to postulate that this damage may be responsible for both R.C.R. and ADP:O reduction in these organells. On the other hand, plant mitochondria posses quite different membrane properties than mammalian ones. For example they utilise the exogenous NADH (SRIVASTAVA and SARKISSIAN 1970), whereas in mammalian mitochondria the inability to oxidaze exogenous NADH may be used as a criterion for well--preparations (CHAPPELL and HANSFORD 1972). It appeared therefore, that in wheat and rye seedlings mitochondria the membrane integrity is not as crucial reason for oxidative phosphorylation efficiency as it is in rat brain and liver mitochondria.

It is concluded that both DDT and DDE affected the electron transfer chain of germinating cereals seedlings mitochondria in a manner similar to that in rat liver mitochondria. Oxidative phosphorylation in rye and wheat seedlings mitochondria is less sensitive to uncoupling action of those pesticides when compared to rat brain and liver mitochondria.

ACKNOWLEDGEMENTS

The excellent technical assistance of Miss ZDZISŁAWA CIEPLIŃSKA is acknowledged with thanks.

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