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SHORT COMMUNICATIONS

Insecticide inhibition of Na-K-ATPase activity*

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IT HAS been claimed that the insecticide DDT selectively inhibits the action of a $(Na^+ + K^+)$-activated, Mg^{2+}-dependent ATP phosphohydrolase [$(Na^+ + K^+)$-ATPase] found in a nerve-ending fraction of the rat brain.[1,2] These investigators used an acetone powder preparation with a low specific activity as the enzyme source. Since this enzyme is a lipoprotein and requires phospholipid for optimal activity, the possibility exists that the natural enzyme may react differently from the acetone-treated enzyme. Therefore, we examined the effects of several insecticides on a rat brain $(Na^+ + K^+)$-ATPase preparation which hydrolyzed approximately 1 and 4 μmoles of ATP per milligram of protein per minute at 23° and 37° respectively.

The enzyme from rat brain was prepared as previously described,[3,4] except that the enzyme was further purified by a sodium iodide treatment.[5] The final residue was resuspended in 4 ml of 10 mM Tris-HCl buffer (pH 7·5), assayed for protein concentration by the method of Lowry *et al.*[6] and stored frozen until ATPase assay. All preparative procedures were carried out at 2°. ATPase activity was estimated by measuring inorganic phosphate (P_i) liberated from ATP. The incubation mixture contained 16 μg of enzyme protein, 5·0 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH 7·5) and various concentrations of inhibitors as indicated, with or without 100 mM NaCl and 15 mM KCl in a total incubation volume of 1·0 ml. Appropriate concentrations of *p,p'*-DDT,† *o,p'*-DDT, *p,p'*-DDE, dieldrin and chlordane in absolute ethanol were added in 20-μl vols. to the incubation mixture to make the final concentrations indicated. After a 5-min preincubation period, Tris-ATP was added to a final concentration of 5·0 mM. The incubation was performed for an additional 10-min period at 37° or 15 min at 23° and 30°. After the reaction was terminated, P_i was determined as described by Bonting *et al.*[7] Mg^{2+}-ATPase activity, assayed in the absence of NaCl and KCl, was subtracted from the total ATPase activity, assayed in the presence of NaCl and KCl, to calculate $(Na^+ + K^+)$-ATPase activity. All assays were performed in duplicates.

All insecticides and the analogues studied inhibited the $(Na^+ + K^+)$-ATPase activity as shown in Table 1. The inhibition was not specific to the active form, *p,p'*-DDT. Less active analogues, namely *o,p'*-DDT and *p,p'*-DDE, were equally potent as enzyme inhibitors. This is a marked contrast to the previous data where DDE was found to be significantly less potent.[1]

In the present study, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and dieldrin are equipotent as inhibitors of $(Na^+ + K^+)$-ATPase, whereas chlordane is significantly more potent. Although a dose–response relationship was not observed with dieldrin at concentrations above 8 μM, this agent was equally potent as other agents at lower concentrations. Thus, we were unable to observe a correlation between the insecticidal action of these agents and their potency as inhibitors of rat brain $(Na^+ + K^+)$-ATPase activity.

Another discrepancy between the present data and previous studies[1,2] is the inhibitory potency of *p,p'*-DDT at different temperatures. These authors observed that the inhibition of $(Na^+ + K^+)$-ATPase by *p,p'*-DDT was greater at 13° than at 23° and less at 37°. In the present study, no significant difference in inhibitory effect of chlordane was observed at 23°, 30° and 37°. Inhibitory effects of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and dieldrin were reduced at lower temperatures, particularly at higher concentrations. The inhibitory effect of *p,p'*-DDT at 13° was not determined in the present study, since the present enzyme preparation had no significant $(Na^+ + K^+)$-ATPase activity at this temperature.

Although it has been reported that $(Na^+ + K^+)$-ATPase can be separated into two different enzymes, one ouabain-sensitive and the other mersalyl-sensitive,[1,2] the $(Na^+ + K^+)$-activated portion of the present enzyme preparation was sensitive to both ouabain and SH-inhibitors. Appropriate

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† Abbreviations used: *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDT, 1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane; and *p,p'*-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene. Chemical names are: dieldrin, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanophthalene; and chlordane, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane.

concentrations of either ouabain or organomercury alone were capable of inhibiting ($Na^+ + K^+$)-ATPase activity completely.

The lack of relationship between the insecticidal activity and the inhibitory effects on ($Na^+ + K^+$)-ATPase observed in the present study does not necessarily rule out the possibility that this is a target enzyme for insecticides. The difference in toxicity among DDT analogues may be because of differences in pharmacological disposition of these agents in the animal body. The lack of negative temperature correlation, *p,p'*-DDT more effective at lower temperature, also does not rule out this possibility. It is probable that the key process to be attacked by insecticides is an enzyme system like ($Na^+ + K^+$)-ATPase which has a reduced activity at lower temperatures. Thus, the toxicity would be greater at lower temperatures because of a smaller safety factor at these temperatures even though the per cent inhibition of the enzyme activity observed *in vitro* is similar at different temperatures.

TABLE 1. THE INHIBITION OF ($Na^+ + K^+$)-ATPASE BY INSECTICIDES*

	Concentration (μM)	Temperature 37°	30° (Per cent inhibition, mean ± S. E.)	23°
Ethanol		6·4 ± 2·3	4·2 ± 3·5	3·6
p,p'-DDT	80	55·3 ± 3·3	49·2 ± 3·7	34·0
	20	42·5		
	8	33·4 ± 3·2	37·1 ± 4·7	30·4
	2	10·5		
o,p'-DDT	80	47·8 ± 6·5	33·8 ± 6·6	36·1
	20	36·9		
	8	32·6 ± 1·6	18·3 ± 7·1	24·1
	2	14·1		
p,p'-DDE	80	57·3 ± 3·4	44·3 ± 7·0	38·8
	20	48·8		
	8	31·8 ± 2·2	30·8 ± 5·4	37·7
	2	12·7		
Dieldrin	80	21·0 ± 3·3	5·2 ± 6·3	9·8
	20	23·6 ± 0·9	7·9 ± 6·9	1·2
	8	29·9 ± 2·3	5·7 ± 8·0	6·3
	2	9·7 ± 6·4	9·3 ± 7·0	0·0
Chlordane	80	82·8 ± 1·6	74·1 ± 6·6	77·6
	8	44·9 ± 3·7	41·0 ± 7·8	44·8

* ATPase activity was assayed in the presence and absence (control) of inhibitors. Per cent inhibition was calculated against the control velocity of each enzyme preparation assayed simultaneously. $N = 3$ where S. E. is shown. Others are the result of a duplicate assay. Control ATPase activity in μmoles P_i per milligram protein per minute: 4·5 ± 0·3 (total) and 4·1 ± 0·3 ($Na^+ + K^+$) at 37°; 2·3 ± 0·1 (total) and 2·1 ± 0·1 ($Na^+ + K^+$) at 30° and 1·0 (total) and 0·87 ($Na^+ + K^+$) at 23°.

In conclusion, the insecticide *p,p'*-DDT inhibited the ($Na^+ + K^+$)-activated, Mg^{2+}-dependent ATP phosphohydrolase activity of a rat brain microsomal fraction. This inhibition was not specific to *p,p'*-DDT and the inhibition of this enzyme system by *p,p'*-DDT was reduced at low incubation temperatures. Its less active analogues, *o,p'*-DDT and *p,p'*-DDE, and a more potent chlorinated insecticide, dieldrin, inhibited the enzyme to a similar degree. Chlordane, another chlorinated insecticide, was the most potent inhibitor of the enzyme of compounds tested. While the present data do not support the hypothesis that the inhibition of this enzyme system *in vitro* is causally related to the insecticidal action of DDT *in vivo*, studies with a ($Na^+ + K^+$)-ATPase from a DDT-sensitive species would be more pertinent to this point.

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Effect of Δ 1-tetrahydrocannabinol* on ATPase activity of rat liver mitochondria

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THE MODE of action of Δ 1-tetrahydrocannabinol, one of the predominant psychoactive components in Cannabis,[1-2] has been the object of intensive study[3-5] after its recent isolation and synthesis.[6] It has been shown[7] that the liver is the major center of accumulation of radioactivity after administration of [^{14}C]-labeled tetrahydrocannabinol and, therefore, it appeared of interest to study the effect of Δ 1-tetrahydrocannabinol (THC) on various parameters in that organ. The work reported below deals with the effect of THC on ATPase activity in rat liver mitochondria.

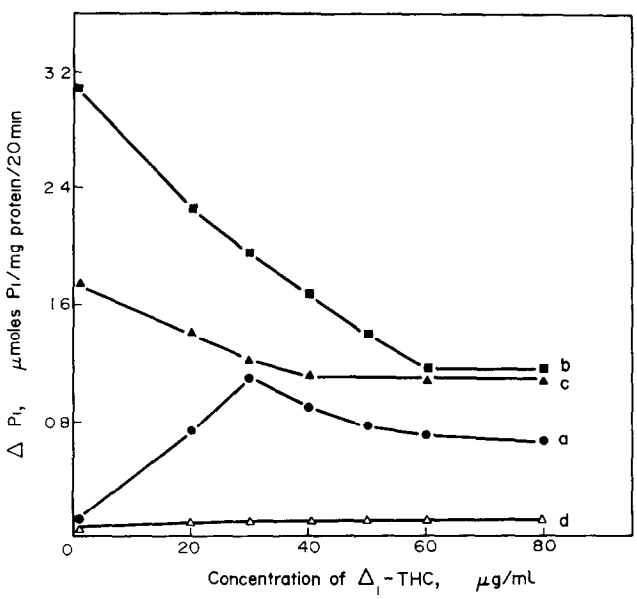

FIG. 1. Effect of Δ 1-THC on ATPase activity. The assay contains in 1 ml: 75 μmoles tris-HCl buffer (pH 7·4); 3·5 μmoles ATP; mitochondrial fraction (0·75 mg protein). a, no addition; b, 0·05 μmoles DNP; c, 0·1 μmoles Ca^{2+}; d, 3 μg oligomycin. In all cases the solutions were made up with sucrose to a final total concentration of 150 μmoles/ml. Temperature 30°. Incubation time 20 min.

* Also designated as Δ 8-tetrahydrocannabinol.