DDT: Inhibition of an ATP-ase in the Rat Brain

by F. MATSUMURA, T. A. BRATKOWSKI, and K. C. PATIL
Department of Entomology, University of Wisconsin, Madison, Wisconsin

To relate the action mechanism of DDT to inhibition of a certain biochemical reaction system, at least three basic requirements must be fulfilled: first, the system must logically be related to the mechanisms of ion transport in the nervous system (1, 2, 3); second, it must be DDT-specific as contrasted to non-active analogs (4); and third, it should be able to form a physicochemical complex with DDT itself. The last requirement is needed, since DDT, by its particular chemical characteristic of extreme stability (5), is expected to form such a complex with the target site to become effective (6).

A system fulfilling these basic requirements has been found in an ATP-ase from the rat brain preparation in this laboratory, and we now report properties of this DDT-sensitive ATP-ase.

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 5, 1969, published by Springer-Verlag New York Inc.

The initial effort was made to localize the source of ATP-ases which are particularly sensitive to DDT within the subcellular components. Fractionation procedures used for collecting various nerve components of the rat brain were those developed by De Robertis and his associates (7). All particulate fractions were examined under an electron microscope to ensure their identities. The particulate, subcellular fractions were resuspended in 0.05 M tris-HCl buffer pH 7. A 0.2 ml aliquot of the enzyme preparation was added to a standard incubation mixture containing 0.1 M KCl (or NaCl) and 1 mM of $MgCl_2$ in 1.8 ml of 0.2 M tris-HCl buffer pH 7. In some cases the system was preincubated at 24° C with ouabain (10^{-4} moles/liter, Nutritional Biochemicals Corp.) or Mersalyl (2.5×10^{-4} moles/liter, Sigma Chemical Co.) for 10 min (added with 18 µl of distilled-deionized water). DDT (or DDE) was added to the system with 18 µl of ethanol (95%) to make the final concentration at 10^{-5} M, and the system was maintained for 10 min. To assay the ATP-ase activity, 1 µmole of ATP (disodium or tris-salt) was added with 0.2 ml of tris-HCl buffer (pH 7), and the system was incubated for 30 min at 24° C. The choice of 24° C rather than 37° C was based upon the well-known phenomenon that DDT-poisoning generally is more pronounced at relatively low temperatures. The total scale of assay procedure for inorganic phosphorus was adjusted to give the final volume of 15 ml (8).

Table 1 illustrates the degrees of inhibition obtained both

TABLE I

DDT and DDE inhibition of ATP-ases in various subcellular nerve components of the rat brain. The data are expressed in % activity remaining after incubation with DDT or DDE for each enzyme source.*

Nerve fractions	% Activity remaining		% Difference (DDT-DDE)	(No. of experiments)
	DDT	DDE		
Supernatant	100	100	0	(1)
Microsome-cell membrane	50.0	50.0	0	(1)
Myelin	54.0	53.0	1.0	(3)
Nerve endings (ACh^+)	63.0	63.0	0	(4)
Nerve endings (ACh^-)	70.0	75.0	-5.0	(5)
Nerve endings (ACh^-)-mitochondrial	81.0	88.0	-7.0	(2)
Nerve ending complex (without synaptic vesicles)	55.7	72.7	-17.0	(6)
Synaptic vesicles	58.1	61.2	-3.1	(3)
Crude nucleus	80.6	79.6	1.1	(1)

* Each enzyme source was diluted to give a hydrolysis rate of approximately 0.1 μmole of ATP per 30 minutes per assay.

by DDT and DDE incubation with various subcellular fractions under the above experimental conditions. It was found that the degree of DDT inhibition exceeded that of DDE only in the fractions containing the nerve endings, particularly in those lacking the synaptic vesicles or acetylcholine. The attempt was made to purify, therefore, the nerve ending complex which lacked the synaptic vesicles by using the osmotic shock treatment (7). The fractions sedimented between 0.9 to 1.2 M sucrose were further treated with acetone at -15° to prepare acetone powder. The acetone powder was suspended in 0.05 M tris-HCl buffer (pH 7) through homogenization. The suspension was centrifuged at 20,000 g for 30 min to obtain the pellet which was resuspended in the same buffer and used for further testing (acetone powder precipitate). It was found that the acetone powder can be stored in a desiccator at 0° C for a period of a few weeks, thus providing a stable enzyme source.

The experiments shown in Table 2 indicated that the enzyme preparation was inhibited by DDT, and to a lesser extent by DDE, in the presence of 0.1 M K^+ and 1 mM Mg^{++}. This finding that the DDT-DDE sensitivity difference remains in the acetone powder precipitate coincides with the data from this laboratory (11) that the fractions containing nerve ending particles are the only ones that show relatively high binding affinity to C^{14} DDT as compared to C^{14} DDE among all nerve components tested, and that more C^{14} DDT binds to the precipitate obtained from the acetone powder suspension

than does C^{14} DDE.

Table 2 also indicates the results of ATP-ase assays in various ion combinations. It is evident from the results that the DDT-sensitive ATP-ase requires the presence of both Mg^{++} and K^{+} (or Na^{+}) as activating ions. Ca^{++} appeared to be a less preferred substitute for Mg^{++}.

Further experiments with varied amounts of monovalent cations indicated that the portions of the enzyme activity inhibited by 10^{-5} M DDT roughly corresponded to those activated by the addition of K^{+} (or Na^{+}) over Mg^{++} ions. For instance, the above enzyme preparation showed approximately 66% of K^{+} (Na^{+})-independent and Mg^{++}-dependent ATP-ase activities which were almost insensitive to either DDT or DDE. The remaining K^{+} (Na^{+}) and Mg^{++}-dependent enzyme(s) appeared to be particularly sensitive to DDT (approx. 90-100% inhibition) in comparison to DDE (approx. 40-50% inhibition). Two possibilities appear to exist to cause such a pattern of DDT inhibition: i.e. either the preparation contained 66% of an independent Mg^{++} ATP-ase as a contaminant, or it mainly consists of one K^{+} (or Na^{+})/Mg^{++} ATP-ase complex (9). In the latter case, DDT must specifically attack the K^{+} (or Na^{+}) site without seriously affecting the Mg^{++} site of the complex.

Various attempts were made, therefore, to eliminate the Mg^{++}-dependent portion of the ATP-ase activity from the acetone powder precipitate preparations by using known inhibitors of ATP-ases. Preincubation of this enzyme preparation with ouabain (final

TABLE II

Effects of DDT and DDE on the activity of the ATP-ases of the acetone powder precipitate under various ion combinations. The results are expressed in % activities against the standard (e.g. $K^+ + Mg^{++} = 100$). The standard activity corresponds to approximately 5 mμmole/mg protein/min of ATP hydrolysis. The values for the nonspecific hydrolysis of ATP were corrected by subtracting the check values (usually 10-20% of the total) obtained by the use of 10^{-3}M EDTA in the incubation system that did not contain any ions.

Ion composition (M)	Control	DDT	DDE	No. of experiments
K^+ (0.1) + Mg^{++} (0.001)	100	63.6	81.8	(7)
Na^+ (0.1) + Mg^{++} (0.001)	114.3	66.2	90.3*	(6)
K^+ (0.1) + Ca^{++} (0.001)	16.9	0	1.7	(2)
Na^+ (0.1) + K^+ (0.1)	2.6	1.6	1.6	(2)
Mg^{++} (0.001)	66.2	61.0	61.0	(6)
Preinhibition with ouabain				
K^+ (0.1) + Mg^{++} (0.001)	61.0	31.1	44.2	(6)
Preinhibition with Mersalyl				
K^+ (0.1) + Mg^{++} (0.001)	14.3	0.8	7.0	(6)

* Corresponding figures for 10^{-5} M DBP (p,p'dichlorophenylbenzophenone) and DDA are 90 and 91%, respectively.

conc. 10^{-4} M), a known specific inhibitor for $Na^+ + K^+/Mg^{++}$ ATP-ases (9) did not entirely eliminate the DDT-DDE difference. Pre-inhibition of the same enzyme source with Mersalyl (final conc. 2.5×10^{-4} M), an inhibitor for Mg^{++}-Ca^{++} ATP-ases (10), markedly reduced the ATP-ase activity due to the Mg^{++}-dependent portion of the enzyme preparation. The remaining enzyme activity could be inhibited by DDT (approx. 95% inhibition) and to a lesser extent by DDE (approx. 50% inhibition). The enzyme, on the other hand, was not seriously affected by other noninsecticidal DDT analogs such as DDA or DBP (p,p'-dichlorophenylbenzophenone). Nor was it inhibited by 5×10^{-4} M (final conc.) of 2,4-dinitrophenol.

The evidence presented in this paper indicates that an K^+ (or Na^+) and Mg^{++} requiring ATP-ase in the rat brain is specifically sensitive to DDT. It has been suggested that ATP-ases in the brain are associated with changes in ion permeability during excitation (10, 12) and with changes in acetylcholine levels in the nervous system (12). It is at least a possibility that inhibition of such protein is causally related to disruption of ion transport mechanisms in the nervous system in vivo. To prove such a relationship, more information is needed concerning the actual role of such ATP-ases in the nervous system. Electrophysiological evidence to correlate the levels of nerve disruption and the degree of inhibition of the ATP-ases by DDT is also necessary.

Acknowledgements

Supported in part by a PHS grant, CC-00252, from the National

Communicable Disease Center, Atlanta, Hatch project 822 and a Research Committee grant from the Graduate School, University of Wisconsin. Approved for publication by the Director of Wisconsin Agricultural Experiment Station. The authors thank Dr. J. N. Telford for his assistance in electronmicroscopic examination of the nerve fractions.

References

1. W. E. DALE, T. B. GAINES, W. J. HAYES and G. W. PEARCE, Science 142, 1474 (1963)
2. T. NARAHASHI and H. G. HAAS, Science 157, 1438 (1967)
3. J. STERNBURG, Ann. Rev. Entomol. 8, 19 (1963)
4. O. GONDA, A. KALUSZYMER, and Y. AVI-DOR, Biochem. J. 73, 583 (1959)
5. F. MATSUMURA and R. D. O'BRIEN, J. Agr. Food Chem. 14, 39 (1966); J. L. EATON and J. STERNBURG, J. Econ. Entomol. 60, 1699 (1967)
6. L. J. MULLINS, Science 122, 118 (1955); F. A. GUNTHER, R. C. BLINN, G. E. CARMAN and R. L. METCALF, Arch. Biochem. Biophys. 50, 504 (1954)
7. E. DE ROBERTIS, Science 156, 907 (1967)
8. B. B. MARSH, Biochem. Biophys. Acta 32, 357 (1959)
9. J. C. SKOU, Physiol. Rev. 45, 597 (1965)
10. S. PUSZKIN, S. BERL, E. PUSZKIN and D. D. CLARKE, Science 161, 170 (1968)
11. H. BRUNNERT and F. MATSUMURA, unpublished data. Presented

by F. Matsumura at the Amer. Chem. Soc. Meet., Atlantic City, N.J. See Abstract, Div. Agr. Food Chem. Paper No. 75, September 10 (1968)

12. M. GERMAIN and P. GROULY, Biochem. Pharmacol. 14, 1815 (1965); K. KADOTA, S. MORI and R. IMAIZUMI, J. Biochem. 61, 424 (1967)