Influence of Some Insecticides on the ATPase of Mouse Liver Mitochondria

by
NANCY B. PAYNE, GENE R. HERZBERG, and JOHN L. HOWLAND
Department of Biology
Bowdoin College
Brunswick, Me. 04011

Effects of insecticides upon both target and non-target organisms often reflect interaction with cellular membranes (MATSUMURA and O'BRIEN, 1966). For instance, in the case of vertebrates, these effects include inhibition of plasma membrane ATPase (adenosine triphosphatase) (DAVIS et al. 1972), increased metabolic transformations by microsomal membranes (REMMER et al. 1967), and disruption of osmotic equilibrium (JANICKI and KINTER 1971). Since mitochondrial energy coupling is localized in the inner mitochondrial membrane and since coupling is highly sensitive to assault on the integrity of that membrane, we have undertaken to examine the action of insecticides on processes associated with mitochondrial energy conservation. The present communication presents data indicating that low concentrations of several chemically-unrelated insecticides stimulate mouse mitochondrial ATPase in a manner similar to that of uncouplers of oxidative ATP synthesis.

Methods

Liver mitochondria were isolated in 250 mM sucrose from mice (strain 129J, Jackson Laboratory, Bar Harbor, Maine). ATPase activity was measured as the release of phosphate from ATP following the procedure of MYERS and SLATER and employing a reaction temperature of 37° and a pH of 7.4. Insecticides were purchased from K and K Laboratories while biochemicals were obtained from Nutritional Biochemicals. Insecticides were added dissolved in dimethyl sulfoxide while comparable amounts of the solvent were included in the controls.

Results and Discussion

The terminal reaction in the oxidative synthesis of ATP may be measured in the reverse direction as ATPase activity, an activity diminished by inhibitors of oxidative phosphorylation and stimulated by uncoupling compounds such as 2;4-dinitrophenol (DNP).

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 6 © 1973 by Springer-Verlag New York Inc.

Table I shows representative experiments indicating that mitochondrial ATPase activity is also stimulated upon addition of small amounts of three structurally-unrelated insecticides.

Table I

Stimulation of mitochondrial ATPase by insecticides

additions	ATPase activity (nmoles/min/mg)
none	10.4
aldrin 62 µM	22.0
allethrin 82 µM	29.8
DDT 64 µM	33.2
DNP 125 µM	54.0
DNP 125 µM + DDT 64 µM	50.2
DNP 125 µM + aldrin 62 µM	56.4

The stimulation of activity produced by insecticides, although substantial, is not as great as that obtained with DNP, a difference that may reflect a limitation due to the solubility of these compounds in water. The lower portion of the table shows that the insecticides do not prevent DNP-induced stimulation, a feature which distinguishes them from certain other uncoupling compounds such as alkyl hydroxynaphthoquinones (HOWLAND, 1967). In other experiments which are not shown, stimulation of ATPase by insecticides is unaffected by alteration of the ionic content of the medium or by lowering the temperature to 20° instead of 37°.

Stimulation of liver mitochondrial ATPase by insecticides suggests that these compounds have the capacity to act in a manner similar to that of uncouplers of oxidative phosphorylation. Such uncoupling would be expected to lead to disruption of cellular energy supply and to contribute to the

toxicity of these compounds. The stimulation of mitochondrial ATPase noted should be considered in contrast to the influence of the same compounds upon the sodium-potassium ATPase of plasma membranes where similar concentrations of the insecticides produce substantial inhibition of activity (DAVIS et al. 1972).

Finally, these results should also be considered in the light of the recent observation of KOMISARENKO and TIULENIEV (1972) that the insecticide DDD stimulates ATPase in mitochondria from the liver and kidney of guinea pigs although, in their study, much higher concentrations of the insecticide were employed than in the present one.

Acknowledgments

This study is supported by NIH grant number GM 17955-02 to J. H. who is also recipient of a U. S. Public Health Service Research Career Development Award number 5 KO4 GM11854.

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