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Effect of Selected Water Toxicants and Other Chemicals Upon Adenosine Triphosphatase Activity *In Vitro*

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The significance of adenosine triphosphatases (ATPase) in controlling high energy metabolic transformations and ion movements in living tissue is well known, and the subject has been extensively reviewed (SKOU 1965, DAHL & HOKIN 1974, SCHWARTZ et al. 1975, YAMAZAKI 1977). These enzymes, particularly Na+/K+ - and Mg++ - activated ATPase, have a relatively high sensitivity to certain classes of pesticides and other pollutants, and it has been strongly suggested that toxicosis from many of these chemicals may develop primarily from ATPase inhibition. Studies have shown that ATPase activity is inhibited by alkyl and aryl organochlorine insecticides (KOCH 1969, CUTKOMP et al. 1971, DAVIS & WEDEMEYER 1971, DESAIAH et al. 1973a, DESAIAH & KOCH 1975a, 1975b, WITHERSPOON & WELLS 1975) and with organochlorine pesticides of the cyclodiene type (CHU & CUTKOMP 1971, WELLS et al. 1974, YAP et al. 1975). Polychlorinated biphenyls were also found to affect enzyme activity (KOCH et al. 1972, NARBONNE et al. 1978), and ATPase inhibition by natural pyrethrins (DESAIAH et al. 1973b) and some pyrethroids was reported (DESAIAH et al. 1975). Certain organo-tin (BYINGTON 1971, DESAIAH et al. 1973a) and organo-mercury compounds (TAYLOR 1963, SCHMIDT-NIELSON 1974) also inhibited ATPases. Concerning inorganic salts, significant effects have been reported for copper, cadmium, silver, mercury, vanadium, zinc, lead, manganese, chromium, iron, and beryllium (RIFKIN 1965, BADER et al. 1970, HEXUM 1974, SHEPHARD & SIMKISS 1978).

The current study was undertaken to improve and simplify a colorimetric procedure for the analysis of ATPase activity and to analyze 19 selected chemicals from structurally different chemical classes, most of which are water pollutants, rate them in accordance with degree of inhibitory effect, and make some judgement regarding which of these agents may cause toxic effects on the living animals through ATPase inhibition or activation. The most accurate assessment of sublethal foreign agent effects on animals ultimately may be derived from precisely measured molecular changes.

MATERIALS AND METHODS

The test chemicals used in this study were (reagent grade): $AgNO_3$, $HgCl_2$, $CuCl_2$, $CdCl_2 \cdot 2\ H_2O$, $AlCl_3$, $NiCl_2 \cdot 6H_2O$ $CoCl_2 \cdot 6H_2O$, NaCl, Na_2S, NaF, CH_3HgCl, malathion, carbaryl,

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lindane, dieldrin, DDT, dicofol, ouabain, and eserine (physostigmine).

The enzyme (1 mg ATPase*/mL) and substrate (2 mM ATP*) were prepared fresh daily in a tris-salt buffer and maintained on ice (50 mM tris at pH 7.0 containing $MgCl_2 \cdot 6H_2O$, 10 mM, KCl, 30 mM, and NaCl, 142 mM). The test chemicals were prepared in water, 50% aqueous ethanol (eserine, malathion, and carbaryl) or in 50% acetone in ethanol (lindane, DDT, dieldrin, and dicofol). In order to determine the control value for the enzymatic hydrolysis of ATP, the enzyme preparation (100 µg/L) was diluted with the tris-salt buffer (500 µL), to which was added the ATP preparation (100 µL). In order to determine the inhibited enzyme acitivity, the toxicant preparation (100 µL) was diluted with the tris-salt buffer (400 µL), to which was added the enzyme (100 µL). The interaction was allowed to proceed for 15 min (at 23° C) with gentle shaking, followed by addition of the substrate (100 µL). The reaction time for enzyme and substrate was 30 min (at 23° C with gentle shaking). Sulfuric acid (300 µL of 6N) was then added to arrest activity and provide the optimum pH for color formation. Tris buffer (50 mM, pH 7.0) was added to bring the volume to 3.5 mL. Phosphate was analyzed according to the malachite green-sodium molybdate method (ALTMANN et al. 1971, MUSZBEK et al. 1977), whereby malachite green was added (500 µL of 0.0185% (w/v) in 0.1% (v/v) aqueous polyvinyl alcohol) to the samples, followed immediately by sodium molybdate (500 µL of 0.1M). After 45 min the absorbance was determined at 625 nm. Net values were obtained by correcting for the respective blank values. The enzyme control blank sample and the enzyme-inhibited blank sample were prepared by adding the enzyme increment after the acid.

The standard curve for phosphate was prepared by using 100 µL increments of Na_3PO_4 in tris-salt buffer (from the range of 10 to 100 µM phosphate) after correcting for phosphate blank values. Protein was determined by the biuret reaction; bovine serum albumin was used as the standard (from 0.1 to 1.0 g%). The analyses were carried out in triplicate and mean values determined. The variability of the assay was about ±5%. Various concentrations of each toxicant chemical were tested to establish a graphic plot of the range from zero to maximum effect and thus obtain a concentration value (in Molarity) to indicate 50% inhibition of activity. These values (I_{50}) are shown in Table 1.

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* Adenosine-5'-triphosphatase, Na+/K+ activated; hog cerebral cortex grade III; activity = 0.3 units/mg protein. Adenosine-5'-triphosphate·2Na·$3H_2O$; crystalline, from equine muscle.

TABLE 1
Effect of chemicals on Na^+/K^+ - ATPase activity (as I_{50} values), in decreasing order of effect

Chemical	I_{50}	Chemical	I_{50}
$AgNO_2$	8.0×10^{-8}	Lindane	3.75×10^{-4}
$HgCl_2$	2.5×10^{-7}	NaF	3.75×10^{-4}
$CuCl_2$	4.75×10^{-7}	$NiCl_2 \cdot 6H_2O$	1.75×10^{-3}
$CdCl_2 \cdot 2\ H_2O$	9.0×10^{-7}	$CoCl_2 \cdot 6H_2O$	3.5×10^{-3}
Dicofol	3.0×10^{-6}	Carbaryl	4.5×10^{-3}
$CH_3\ HgCl$	3.75×10^{-6}	Malathion	9.5×10^{-3}
Ouabain	3.0×10^{-5}	Eserine	0
DDT	1.5×10^{-4}	Na_2S	0
Dieldrin	2.0×10^{-4}	NaCl	0
$AlCl_3$	2.5×10^{-4}		

RESULTS AND DISCUSSION

Salts of Ag^{+1}, Hg^{+2}, Cu^{+2}, and Cd^{+2} were the most inhibiting chemicals to the enzyme (Table 1). These results suggest that ATPase, like so many other enzymes, has a particular sensitivity to those transition metal cations with a relatively high electronegativity.

Of the pesticides studied, the arylorganochloride pesticide dicofol was the strongest inhibitor, followed by DDT, dieldrin, lindane, carbaryl, and malathion. These data in general agree with *in vivo* experiments by KOCH (1969) and CUTKOMP et al. (1971), which showed that the organochloride pesticides are inhibiting and more so than the organophosphate pesticides such as malathion and the carbamates such as carbaryl. The carbamate eserine was unreactive. Ouabain, considered a potent inhibitor of Na^{+1}/K^{+1} - ATPase, was about 375 times less reactive than Ag^{+1}. An intermediate effect on activity was found for Al^{+3}, F^{-1}, Ni^{+2}, and Co^{+2}. No measurable effect was discernible for S^{-2} and NaCl.

The toxic effect of heavy metal salts and some of the organometals and organochlorine pesticides might be caused by interruptions of energy-mobilizing and sensory-transmitting systems, of which ATP and its associated enzymes are an integral part. It seems unlikely that the organophosphate and carbamate pesticides exert a primary deleterious effect through ATPase inhibition, although a long exposure time and a high degree of bioaccumulation, coupled with a low rate of metabolic degradation could ultimately bring about an effect. These results provide some correlation with previously published data and focus further attention upon the probable involvement of ATPases in the generation of a toxic state in aquatic animals caused by some chemicals.

Further *in vitro* and *in vivo* studies of ATPase and other enzymes are needed in order to clarify the role that enzymes may have in biological interactions involving toxic environmental pollutants.

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