Organochlorine Insecticide, Herbicide and Polychlorinated Biphenyl (PCB) Inhibition of NaK-ATPase in Rainbow Trout

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The current widespread presence of chlorinated insecticides, polychlorinated biphenyls (PCB's) and herbicides in world waterways has elicited much interest in the mechanisms of their toxicity in fishes. Inhibition of Na^+,K^+-activated adenosinetriphosphatase (NaK-ATPase) and Mg^{++}-dependent ATPase (Mg-ATPase) by DDT, endosulfan and dicofol has been demonstrated in gill, brain and kidney microsomes of rainbow trout (1,2). Intestinal and gill ATPases in marine teleosts were recently reported to be sensitive to organochlorines (3). Cutkomp et al (4) noted inhibition of NaK-ATPase and Mg-ATPase in bluegill brain, liver, muscle and kidney by DDT and related chlorinated hydrocarbons. Inhibition of ATPases by PCB's has been recently shown in bluegill kidney, brain and liver (5). In the present study, we have further examined the NaK-ATPase enzyme system in trout gill as a site for the possible toxicity of selected organopolychlors, i.e., chlorinated insecticides, herbicides and PCB's.

MATERIALS AND METHODS

ATPase activities of a heavy microsomal fraction of gill homogenate from yearling rainbow trout (Salmo gairdneri) were determined as previously described (2,6). NaKMg-ATPase was assayed in the presence of 3mM Tris-ATP, 100mM NaCl, 15mM KCl, 3mM $MgCl_2$ and 30 mM Tris-HCl at pH 7.5 during incubation for 15 min. at 35° C (Q_{10} = 1.5 from 25-35°C). Mg-ATPase activity was measured under the same conditions with the exception that 115 mM choline chloride replaced the Na^+ and K^+. NaK-ATPase activity was calculated as the difference between NaKMg-ATPase and Mg-ATPase values. Under these conditions, ATP hydrolysis was a linear function of time and enzyme concentration.

Fourteen polychlorinated insecticides were tested for possible inhibition of trout gill ATPase: chlordane, heptachlor, DDD, aldrin, toxaphene, DDT, strobane, DDE, perthane, methoxychlor, lindane, dieldrin, endrin and mirex. Two PCB's, Arochlor 1242 and Arochlor 1254, as well as two herbicides, 2,4-D and 2,4,5-T, were similarly investigated. Samples used were gas chromatographic standards (Polyscience) except the PCB's which were supplied by Monsanto. All compounds tested were dissolved in 20 µl of dimethylsulfoxide (DMSO) and were added to the 2 ml ATPase assay to yield final concentrations of 10^{-4} of 10^{-5} M.

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For compounds of uncertain molecular weight, such as toxaphene, concentrations were expressed on a ppm basis (4ppm = 10^{-5} M assuming M.W. = 400). DMSO (20 µl) alone did not alter normal ATPase activity.

RESULTS AND DISCUSSION

Control values for specific activity (µM P_i per mg protein in 15 min) in gill were 29.4 ± 3.7 (mean ± S.E., n=4) for NaKMg-ATPase and 11.5 ± 1.1 for Mg-ATPase with a calculated difference of 17.8 ± 2.7 for NaK-ATPase.

ATPase activity in the presence of organopolychlors is presented in Table 1. Inhibition of NaKMg-ATPase by the insecticides at either 10^{-4} M or 40 ppm ranged from 77% for chlordane to essentially zero for mirex. Six of the insecticides (from chlordane through DDT) inhibited NaKMg-ATPase at a substantially lower concentration (either 10^{-5} M or 4 ppm). At 40 ppm, the two PCB's inhibited by only 26% (Arochlor 1254) and 36% (Arochlor 1242) while the herbicides produced no significant decrease in NaKMg-ATPase activity at 10^{-4} M. Organopolychlors did not selectively inhibit NaK-ATPase activity, i.e., a general depression of NaKMg-ATPase, NaK-ATPase and Mg-ATPase activities was observed. In fact, the highest inhibition figures were most often associated with Mg-ATPase.

The degree of turbidity (lindane > toxaphene > endrin > aldrin) seen with certain organopolychlors in the final ATPase assay, a function of their water insolubility (7), was not related to the extent of ATPase inhibition. However, there did appear to be a relationship between ATPase inhibition and the solubility (7) of these organopolychlors in organic solvents (in benzene: heptachlor > DDT > lindane > mirex and in aliphatic hydrocarbons: heptachlor > methoxychlor > endrin). Thus the lipid (membrane) solubility of these agents may be a factor in the magnitude of their inhibition of ATPase.

In relating the known *in vivo* lethality (8,9) of these compounds to the present results, the insecticides were both more toxic and more effective ATPase inhibitors than were the herbicides. Intermediate values for both toxicity and enzyme inhibition were observed for PCB's. However, within the insecticide group, toxicity and ATPase inhibition did not correlate, e.g., dieldrin and endrin which were very weak enzyme inhibitors are among the most toxic *in vivo*.

In addition, in recent *in vivo* experiments (10), we failed to demonstrate inhibition of trout ATPases following oral administration of DDT. Single DDT doses of 5 mg/kg and 10 mg/kg produced death within 36 hours in 8% and 64%, respectively, of a population of 50 trout; no deaths occurred in a sham-dosed control population. Gas chromatographic analysis of gills, brain and kidney revealed maximum levels of DDT of 1-2 ppm. In these

Table 1

Inhibition of ATPase activity in trout gill microsomes by insecticides, PCB's and herbicides

	molarity (or ppm)	% inhibition of ATPase$^{\Phi}$ NaKMg	NaK	Mg
Insecticides				
chlordane	10^{-4}	77. ± 1.6	77. ± 3.7	71. ± 1.6
chlordane	10^{-5}	18. ± 1.8	7.2± 3.2 *	33. ± 2.1
heptachlor	10^{-4}	67. ± 1.3	67. ± 8.2	70. ± 8.2
heptachlor	10^{-5}	13. ± 1.3	1.3± 0.6 *	31. ± 4.6
DDD	10^{-4}	63. ± 1.4	68. ± 4.9	58. ± 6.6
DDD	10^{-5}	25. ± 0.6	13. ± 0.4	44. ± 0.8
aldrin	10^{-4}	54. ± 4.0	45. ± 8.2	68. ± 7.3
aldrin	10^{-5}	8.5± 1.6	1.0± 1.6 *	23. ± 1.8
toxaphene	40 ppm	49. ± 4.2	36. ± 6.0	66. ± 1.4
toxaphene	4 ppm	8.6± 0.9	- 1.0± 0.0 *	24. ± 3.2
DDT	10^{-4}	40. ± 4.2	29. ± 2.9	57. ± 8.2
DDT	10^{-5}	23. ± 3.8	15. ± 4.0 *	34. ± 5.4
strobane	40 ppm	37. ± 5.8	20. ± 6.7 *	61. ± 6.6
DDE	10^{-4}	21. ± 3.0	5.7± 2.9 *	46. ± 5.2
perthane	10^{-4}	19. ± 0.9	3.3± 0.7	43. ± 2.7
methoxychlor	10^{-4}	19. ± 1.0	8.9± 1.9	32. ± 4.0
lindane	10^{-4}	18. ± 2.3	11. ± 3.4 *	28. ± 2.6
dieldrin	10^{-4}	12. ± 2.6	8.5± 4.3 *	18. ± 0.3
endrin	10^{-4}	4.0± 2.0 *	- 0.7± 0.3 *	12. ± 2.3
mirex	10^{-4}	3.7± 3.3 *	1.0± 3.6 *	1.0± 3.1 *
PCB's				
1242	40 ppm	36. ± 1.9	25. ± 3.2	53. ± 2.6
1242	4 ppm	13. ± 1.2	7.6± 1.8 *	22. ± 0.9
1254	40 ppm	26. ± 4.8	17. ± 3.7	40. ± 7.2
1254	4 ppm	15. ± 4.1 *	7.5± 4.4 *	25. ± 5.0
Herbicides				
2,4-D	10^{-4}	2.1± 0.6 *	4.1± 0.5	- 0.2± 0.8 *
2,4,5-T	10^{-4}	1.6± 0.3	5.1± 0.8	- 1.1± 0.3 *

Φ - NaK-ATPase activity (mean ± S.E. with n=3) was calculated as the difference between activity in the presence of 100 mM Na, 15 mM K and 3 mM Mg (NaKMg-ATPase) and that in the presence of 115 mM choline and 3 mM Mg (Mg-ATPase)

* - not significant at 0.05 level

tissues, there was no difference in ATPase activity between the DDT-treated and control fish 24 hours following capsule administration.

In view of the above limitations in relating enzyme inhibition to *in vivo* toxicity, further studies of the NaK-ATPase enzyme system as a possible site for the toxicity of organopolychlors in fishes must be interpreted with caution.

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