THE HYDROLYSIS OF ADENOSINETRIPHOSPHATE BY BLUEGILL LIVER MITOCHONDRIA IN THE PRESENCE OF 2,4-DICHLOROPHENOXYACETIC ACID DERIVATIVES

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ABSTRACT. — The effects of the sodium salt, the dimethylamine salt, the butyl ester, the butoxy-ethanol ester, the ethyl ester, the isopropyl ester, the isooctyl ester, the propyleneglycolbutyl-ether ester, and the 2-ethyl-hexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) on the hydrolysis of adenosinetriphosphate (ATP) by the bluegill (*Lepomis macrochirus*) liver mitochondria in the presence of five metals were investigated. The butyl and isopropyl esters increased the hydrolysis of ATP in the presence of manganese, magnesium, and calcium; however, the isopropyl ester was more effective than the butyl ester in the presence of magnesium and calcium. The butyl ester did not appreciably alter the hydrolysis of ATP in the presence of cadmium and zinc, but the isopropyl ester inhibited the hydrolysis of ATP in the presence of both metals. The dimethylamine salt was the least effective of all the derivatives investigated, and the other derivatives varied in their effect on the hydrolysis of ATP in the presence of the various metals.

The hydrolysis of adenosinetriphosphate (ATP) by bluegill (*Lepomis macrochirus*) liver mitochondria was recently reported (Hiltibran 1966) and was part of an investigation to determine the effect of pollutants on fishes.

The data of Hughes and Davis (1963) and Lawrence (1962) indicated that some derivatives of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) were more toxic to bluegills than were other 2,4-D derivatives. Similar data had been obtained in our laboratory and recently was reported (Hiltibran 1967*a*).

Since some of the 2,4-D derivatives had been shown to be more toxic to small bluegills than other 2,4-D derivatives, it would appear that the more toxic 2,4-D derivatives might alter some biochemical activity and these changes could be estimated. An investigation of the effects of nine 2,4-D derivatives on the hydrolysis of ATP by the mitochondria from bluegill liver in the presence of cadmium, zinc, manganese, magnesium and calcium was initiated. In addition, the effects of the nine derivatives of 2,4-D on succinic acid oxidase and the alpha-Ketoglutaric acid oxidase enzyme complexes from the mitochondria of bluegill liver were investigated and are summarized elsewhere (Hiltibran 1968).

MATERIALS AND METHODS

Native wild bluegills were held in the laboratory in aerated aquaria at 25° C and all enzymatic assays were conducted

at the same temperature. Procedures for the preparation of the mitochondria, for estimating the rate of release of inorganic phosphate from ATP, and for estimating the nitrogen content of the mitochondrial preparations have been reported (Hiltibran and Johnson 1965). The amounts of inorganic phosphate released from the ATP were converted to micromoles of ATP hydrolyzed per hour per milligram of tissue nitrogen. The data are the average change observed from three or more experiments and have been corrected for endogenous activity and the effects of the solvent. The technical grade or pure derivatives were obtained from various manufacturers and were used without further purification. Redistilled ethyl alcohol and acetone were used as solvents.

Results and Discussion

The effects of various 2,4-D derivatives on the cadmium-enhanced hydrolysis of ATP are summarized in

Table 1.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Cadmium

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	—38	—37	—48
# exp.	(4)	(5)	(6)
Range	((—) 8 — (—) 90)	((—) 12 — (—) 76)	((—) 3 — (—) 104)
Dimethylamine "salt"	±7	±19	±22
# exp.	(3)	(3)	(3)
Range	((—) 2 — (+) 15)	((—) 6 — (+) 37)	((+) 24 — (—) 40)
Butyl ester	±6	±11	±3
# exp.	(4)	(3)	(3)
Range	((+) 8 — (—) 14)	((—) 13 — (+) 15)	((—) 2 — (+) 6)
Butoxy—ethanol ester	—11	—19	—13
# exp.	(3)	(3)	(5)
Range	((—) 10 — (—) 14)	((—) 8 — (—) 39)	(0 — (—) 39)
Ethyl ester	.....	—41	—65
# exp.	.....	(3)	(3)
Range	.....	((—) 13 — (—) 65)	((—) 35 — (—) 100)
Isopropyl ester	—15	—27	—45
# exp.	(4)	(7)	(3)
Range	((—) 6 — (—) 37)	((—) 6 — (—) 54)	((—) 22 — (—) 71)
Isooctyl ester	±11	±30	±26
# exp.	(4)	(6)	(4)
Range	((+) 7 — (—) 13)	((+) 21 — (—) 42)	((+) 20 — (—) 43)
PGBE ester	—14	—20	—29
# exp.	(4)	(4)	(3)
Range	((—) 5 — (—) 33)	((—) 9 — (—) 31)	((—) 15 — (—) 51)
2-ethyl-hexyl ester	±3	—23	—28
# exp.	(4)	(4)	(3)
Range	((—) 13 — (+) 5)	((—) 13 — (—) 28)	((—) 22 — (—) 35)

TABLE 2.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Zinc.

2, 4-D Derivatives	μmoles of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles/ml/ATP/hr/mgN		
Na salt	—25	—34	—35
# exp.	(3)	(4)	(4)
Range	((—) 24 — (—) 28)	((—) 27 — (—) 39)	((—) 2 — (—) 64)
Dimethylamine salt	±10	±13	±21
# exp.	(3)	(5)	(5)
Range	((+) 9 — (—) 20)	((+) 5 — (—) 28)	((+) 2 — (—) 47)
Butyl ester	±13	±7	±15
# exp.	(7)	(6)	(6)
Range	((+) 0.6 — (—) 28)	((—) 2 — (+) 17)	((—) 22 — (+) 21)
Butoxy-ethanol ester	—17	—14	—17
# exp.	(3)	(4)	(4)
Range	((—) 8 — (—) 31)	((—) 7 — (—) 18)	((—) 4 — (—) 25)
Ethyl ester	.....	—22	—46
# exp.	.....	(3)	(3)
Range	.....	((—) 5 — (—) 35)	((—) 38 — (—) 54)
Isopropyl ester	—10	—21	—7
# exp.	(5)	(5)	(4)
Range	((—) 0 — (—) 23)	((—) 6 — (—) 36)	((—) 4 — (—) 11)
Isooctyl ester	—10	—15	—21
# exp.	(5)	(6)	(5)
Range	(0.3 — (—) 22)	((—) 2 — (—) 59)	((—) 8 — (—) 53)
PGBE ester	±5	±18	±18
# exp.	(5)	(8)	(5)
Range	((—) 6 — (+) 8)	((—) 33 — (+) 27)	((—) 45 — (+) 13)
2-ethyl-hexyl ester	±7	±10	±3
# exp.	(5)	(5)	(4)
Range	((—) 3 — (+) 20)	((—) 5 — (+) 15)	((+) 0.2 — (—) 6)

Table 1. The dimethylamine salt and the isooctyl ester did not alter the hydrolysis of ATP. Six derivatives inhibited the hydrolysis of ATP, and the butyl ester only at high levels inhibited the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of zinc are summarized in Table 2. The butyl ester, PGBE ester and the 2-ethyl-hexyl ester did not alter the hydrolysis of ATP. The dimethylamine salt at high levels increased the hydrolysis of ATP. The other derivatives at all levels investigated inhibited the hydrolysis of ATP.

The effects of 2,4-D derivatives on the hydrolysis of ATP in the presence of manganese are summarized in Table 3. The sodium salt and the butoxy-ethanol ester inhibited the hydrolysis of ATP. The dimethylamine

salt, the ethyl ester, and the isooctyl ester did not alter the hydrolysis of ATP. The butyl ester, the isopropyl ester, the PGBE ester and the 2-ethyl-hexyl ester at high levels increased the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of magnesium are summarized in Table 4. The sodium salt, the dimethylamine salt, the butoxy-ethanol ester and the ethyl ester did not alter the hydrolysis of ATP. The butyl ester, isopropyl ester and 2-ethyl-hexyl ester at all levels utilized increased the hydrolysis of ATP, whereas the isooctyl ester and the PGBE ester at high levels increased the hydrolysis of ATP.

The effects of the 2,4-D derivatives on the hydrolysis of ATP in the presence of calcium are summarized in

TABLE 3.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Manganese.

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	—27	—27	—38
# exp.	(3)	(3)	(3)
Range	((—) 17 — (—) 37)	((—) 14 — (—) 37)	((—) 22 — (—) 54)
Dimethylamine salt	±12	±7	±6
# exp.	(3)	(3)	(3)
Range	((—) 6 — (+) 22)	((—) 2 — (+) 11)	((—) 1 — (+) 8)
Butyl ester	±3	+23	+45
# exp.	(4)	(4)	(4)
Range	((—) 5 — (+) 6)	((+) 8 — (+) 33)	((+) 12 — (+) 93)
Butoxy-ethanol ester	—13	—22	—33
# exp.	(3)	(7)	(3)
Range	((—) 4 — (—) 28)	((—) 8 — (—) 30)	((—) 20 — (—) 46)
Ethyl ester	.....	±21	±17
# exp.	.....	(5)	(3)
Range	.....	((—) 20 — (+) 45)	((+) 2 — (—) 42)
Isopropyl ester	±18	+35	+47
# exp.	(4)	(6)	(4)
Range	((—) 4 — (+) 59)	((+) 2 — (+) 105)	((+) 36 — (+) 68)
Isooctyl ester	±7	±16	±18
# exp.	(4)	(7)	(4)
Range	((—) 2 — (+) 10)	((+) 1 — (—) 23)	((—) 3 — (+) 50)
PGBE ester	±10	±18	+21
# exp.	(4)	(7)	(4)
Range	((—) 13 — (+) 13)	((—) 30 — (+) 26)	((+) 14 — (+) 27)
2-ethyl-hexyl ester	±8	±8	+40
# exp.	(4)	(5)	(3)
Range	((—) 2 — (+) 16)	((—) 3 — (+) 2)	((+) 10 — (+) 63)

TABLE 4.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Magnesium.

2, 4-D Derivative	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	±5	±8	±7
# exp.	(4)	(6)	(4)
Range	((—) 16 — (+) 12)	((—) 17 — (+) 7)	((+) 5 — (—) 10)
Dimethylamine salt	±17	±15	±19
# exp.	(3)	(4)	(4)
Range	((+) 3 — (—) 42)	((+) 4 — (—) 50)	((+) 3 — (—) 56)
Butyl ester	+33	+41	+48
# exp.	(4)	(5)	(4)
Range	((+) 15 — (+) 54)	((+) 23 — (+) 67)	((+) 29 — (+) 82)
Butoxy-ethanol ester	±12	±20	±13
# exp.	(3)	(5)	(4)
Range	((—) 2 — (+) 18)	((—) 3 — (+) 61)	((—) 3 — (+) 28)
Ethyl ester		±3	±6
# exp.		(4)	(4)
Range		((—) 1 — (+) 10)	((—) 4 — (+) 16)
Isopropyl ester	+49	+51	+28
# exp.	(4)	(4)	(4)
Range	((+) 31 — (+) 74)	((+) 36 — (+) 82)	((+) 5 — (+) 45)
Isooctyl ester	±4	±2	+19
# exp.	(5)	(4)	(4)
Range	((—) 8 — (+) 2)	((—) 2 — (+) 3)	((+) 6 — (+) 29)
PGBE ester	±10	±10	+36
# exp.	(4)	(6)	(4)
Range	((—) 4 — (+) 17)	((—) 8 — (+) 20)	((+) 6 — (+) 67)
2-ethyl-hexyl ester	+25	+19	+50
# exp.	(4)	(3)	(3)
Range	((+) 2 — (+) 54)	((+) 2 — (+) 66)	((+) 34 — (+) 66)

Table 5. The sodium salt, the dimethylamine salt and the isooctyl ester did not alter the hydrolysis of ATP. The isopropyl ester at all levels investigated increased the hydrolysis, whereas the other esters only at high levels increased the hydrolysis of ATP.

Previously reported micromoles of ATP hydrolyzed per hour per milligrams of tissue nitrogen are as follows: cadmium 90, zinc 70, manganese 50, magnesium 28, calcium 24 (Hiltibran, 1966). Therefore, the maximum inhibition of the hydrolysis of ATP by the ethyl ester in the presence of cadmium and zinc was approximately 70 percent. Butoxy-ethanol ester inhibited the hydrolysis of ATP in the presence of manganese approximately 60 percent. The maximum increase in the hydrolysis of

ATP in the presence of magnesium by low levels of the butyl ester and the isopropyl ester was approximately 120 and 150 percent respectively. The maximum increase of the hydrolysis of ATP by the butyl ester in the presence of calcium was about 100 percent.

Since both the butyl ester and the isopropyl ester at low levels (1.5 μmoles) altered the hydrolysis of ATP in the presence of magnesium, the effect of 0.15 and 0.25 μmoles of these esters on the hydrolysis of ATP were investigated. The isopropyl ester at a level of 0.15 μmoles increased the hydrolysis approximately 30 percent, whereas the butyl ester was not effective. Both esters increased the release of inorganic phosphate from ATP at the 0.25 μmoles level and above.

TABLE 5.—The Effects of 2, 4-D Derivatives on the Hydrolysis of ATP in the Presence of Calcium.

2, 4-D Derivatives	μmoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in μmoles ATP/hr/mgN		
Na salt	±8	±4	±5
# exp.	(3)	(5)	(5)
Range	((—) 3 — (+) 12)	((—) 12 — (+) 2)	((—) 16 — (+) 2)
Dimethylamine salt	±6	±8	±3
# exp.	(3)	(3)	(4)
Range	((—) 1 — (+) 14)	((—) 6 — (+) 14)	((+) 4 — (—) 9)
Butyl ester	±4	±15	+25
# exp.	(6)	(5)	(4)
Range	((—) 1 — (+) 10)	((—) 3 — (+) 49)	((+) 3 — (+) 50)
Butoxy-ethanol ester	±5	±6	+10
# exp.	(3)	(5)	(3)
Range	((—) 0.2 — (+) 13)	((—) 0.7 — (+) 26)	((+) 8 — (+) 12)
Ethyl ester	.....	±5	+10
# exp.	.....	(3)	(3)
Range	.....	((—) 2 — (+) 10)	((+) 7 — (+) 11)
Isopropyl ester	+13	+16	+17
# exp.	(4)	(4)	(3)
Range	((+) 2 — (+) 31)	((+) 4 — (+) 45)	((+) 11 — (+) 29)
Isooctyl ester	±2	±6	±2
# exp.	(4)	(5)	(4)
Range	((—) 5 — (+) 1)	((—) 6 — (+) 8)	((—) 1 — (+) 4)
PGBE ester	±3	±7	+14
# exp.	(3)	(6)	(3)
Range	((+) 2 — (—) 6)	((—) 8 — (+) 13)	((+) 10 — (+) 17)
2-ethyl-hexyl ester	±3	±4	+14
# exp.	(4)	(4)	(3)
Range	((—) 1 — (+) 6)	((—) 7 — (+) 4)	((+) 8 — (+) 18)

The data also indicated that various derivatives of 2,4-D can alter the hydrolysis of ATP in the presence of various metals to different extents and in different ways. The data also indicates that high levels of many of the derivatives used in this investigation can alter the hydrolysis of ATP.

The biochemical importance of the hydrolysis of ATP in the presence of cadmium, zinc, manganese and calcium is not known. Magnesium appears to be involved in the conversion of energy produced by the oxidation of substrates into useful energy, presumably via ATP or other phosphate intermediates. Therefore, the effects of the various 2,4-D derivatives on the hydrolysis of ATP in the presence of magnesium may be important.

The effect of cadmium and zinc on the hydrolysis of ATP cannot be discounted. In the previous studies (Hiltibran 1965, 1967b) cadmium and zinc at very low levels inhibited the oxygen uptake in the presence of succinate and alpha-ketoglutarate and caused large increases in the phosphate content of the reaction vessels, i.e. greater than the phosphate contents observed when oxygen uptake was inhibited by sodium cyanide or other inhibitors which did not alter the hydrolysis of phosphate intermediates. These data indicated that cadmium and zinc may have had a two-fold action, i.e. one on the inhibition of oxygen uptake, and a second one on the hydrolysis of phosphate intermediates. The latter was confirmed when it was found that cadmium and zinc increased the hydrolysis of ATP (Hiltibran 1966). It would appear, therefore, that the greater toxicity of cadmium to bluegills may be due to the greater disruption of energy production by cadmium than the disruption of energy production by zinc. Similar observations are available for manganese and calcium although the effects of these metals on the oxygen uptake and hydrolysis of phosphate intermediates were less severe. It would appear that the isopropyl ester could disrupt energy production to a greater extent than the other 2,4-D derivatives. This would appear to correlate with the greater toxicity of the isopropyl ester to bluegills than the toxicity of the other 2,4-D derivatives to bluegills (Hughes and Davis 1963).

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