EFFECTS OF THE CHLOROGROUPS OF PHENOXY COMPOUNDS ON THE OXIDATIVE PHOSOHORYLATION

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ABSTRACT.—The effects of the sodium salts of phenoxyacetic acid, phenoxypropionic acid, 2-chlorophenoxyacetic acid (2-CPA), 4-chlorophenoxyacetic acid, (4-CPA), 2,4-dichlorophenoxpropionic acid, dichlorprop, 4(2,4-dichloro phenoxy) butyric acid (2,4-DB) on the oxygen and phosphate uptake by the bluegill (*Lepomis macrochirus*) liver mitochondria in the presence of succinate and alphahitreglutarate as substrates were investigated and compared with the effects of 2,4- dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-(2,4,5-trichlorophenoxy) propionic acid, silvex. The phenoxyacetic and phenoxypropionic acids did not alter the oxygen uptake in the presence of either substrate. The 2-CPA inhibited oxygen uptake more than the 4-CPA derivative, and the dichloro-substituted phenoxyacetic acid, 2,4-D inhibited oxygen uptake to about the same extent as the 4-CPA. The oxygen uptake was more severely inhibited by the trichloro derivative (2,4,5-T) in the presence of both substrates. The dichlorprop inhibited oxygen uptake more than the trichloro derivative, silvex.

In the presence of succinate, the phenoxyacetic and phenoxypropionic acids increased phosphate uptake, but in the presence of alpha-ketoglutarate, the phenoxyacetic acid and phenoxypropionic derivatives decreased phosphate uptake. The substituted chloro groups did not appear to have an appreciable affect on phosphate metabolism.

While investigating the effects of phenoxy herbicides on energy production by bluegill liver mitochondria, we found that various phenoxy derivatives altered oxidative phosphorylation in the presence of either succinate or alpha-hitoglutarate as substrates. Many of these derivatives altered the hydrolysis of ATP. A specific pattern of effects could not be determined, but the data suggested that the chloro-substituted phenoxy compounds inhibited oxygen uptake by bluegill liver mitochondria, but the effects on oxygen uptake were altered by the various derivatives substituted on the acetic acid.

The sodium salts of phenoxyacetic acid and phenoxypropionic acid did not inhibit oxygen uptake by bluegill liver mitochondria, whereas the sodium salts of the chloro-substituted phenoxy compounds altered the uptake of oxygen. This finding suggested that the uptake of oxygen by bluegill liver mitochondria was influenced by the position and number of chloro groups substituted on the phenoxy ring.

METHODS

Native wild bluegills from various bodies of water in central Illinois were held approximately two weeks at 25° C in aerated laboratory aquaria prior to use. Procedures for the preparation of the liver mitochondria, for estimating the rates of oxygen and phosphate metabolism by conventional Warburg and spectrophotometric techniques, respectively, for estimating the rate of release of inorganic phosphate from the ATP, and for estimating the nitrogen content of the mitochondrial preparations, have been reported (Hiltibran and Johnson 1965). Oxygen data were converted to microliters of O_2 per hour per milligram of tissue nitrogen (ul O_2/hr/mg N), and all phosphate data were converted to micromoles of PO_4 per hour per milligram for tissue nitrogen (mircomoles/PO_4/hr/mg N). All data have been corrected for endogenous enzyme activity, and the results presented are the average values from three or more experiments. The sodium salts were

prepared from reference samples, 2,4,5-T and silvex or reagent grade phenoxyacetic acid, phenoxypropionic, 2-CPA, and 4-CPA.

Results and Discussion

The effects of phenoxy compounds on the oxygen and phosphate uptake by bluegill liver mitochondria in the presence of succinate as substrate are summarized in Table 1. The sodium salts of the phenoxyacetic or phenoxypropionic acid did not alter oxygen uptake. The phenoxy compound with one chloro group at the 2 position, 2-chlorophenoxyacetic acid (2-CPA) inhibited oxygen uptake approximately 38 percent, and inhibited oxygen uptake more than the phenoxy compound with one chloro group at the 4 position, 4-chlorophenoxyacetic acid (4-CPA). The phenoxy compound with the chloro groups at the 2 and 4 positions 2,4-dichlorophenoxyacetic acid (2,4-D) inhibited oxygen uptake less than the 2-CPA, but more than the 4-CPA. The phenoxy compound with three chloro groups, at the 2, 4 and 5 positions (2,4,5-T) inhibited oxygen uptake more than the other chloro phenoxy compounds used in this investigation.

Table 1.—Effects of chlorophenoxy compounds on succinic oxidase.

		CONTROL		EXPERIMENTAL	
Derivative	g x 10^{-4} per ml Reaction medium	ulO_2/hr/ mg N (Ave.)	umoles PO_4/hr/ mg N (Ave.) (1)	μl O_2/hr/ mg N (Ave.)	μmoles PO_4/hr/ mg N (Ave.) (1)
Phenoxyacetic	1.5	253	24	253	34
# exp.		(4)	(4)	(5)	(5)
Range		(175-328)	(5-44)	(211-310)	(25-47)
2-chloro	3.4	205	41	125	24
# exp.		(4)	(4)	(4)	(4)
Range		(116-397)	(20 - 74)	(73-256)	(7-46)
4-chloro	3.4	205	41	152	26
# exp.		(4)	(4)	(4)	(4)
Range		(116-397)	(20 - 74)	(51-308)	(16-35)
Dichloro	3.7	310	37	224	10
# exp.		(3)	(3)	(5)	(5)
Range		(300-321)	(29 - 51)	(188-255)	(4 -24)
Trichloro	4.3	272	37	104	7
# exp.		(5)	(5)	(5)	(5)
Range		(250-296)	(29 - 45)	(1-159)	(5-10)
Phenoxypropionic	1.7	253	24	245	27
# exp.		(4)	(4)	(5)	(5)
Range		(175-328)	(5 - 44)	(215-320)	(22-34)
Dichloro	2.4	342	47	153	20
# exp.		(3)	(3)	(6)	(6)
Range		(297-379)	(2 - 42)	(45-266)	(14-45)
Trichloro	4.5	272	37	114	8
# exp.		(5)	(5)	(5)	(5)
Range		(250-296)	(29 -45)	(82-207)	(5-15)
Phenoxybutyric dichloro	2.5	155	36	11	+45 (2)
# exp.		(8)	(4)	(5)	(5)
Range		(71 -201)	(15-38)	(2-25)	(24-66)

(1) Decrease in phosphate content of reaction media. (2) Increase in phosphate content of reaction media.

The dichloro phenoxypropionic acid, 2(2,4-dichlorophenoxy)propionic acid (dichlorprop) and the trichloro phenoxy compound, 2(2,4,5-trichlorophenoxy)propionic acid (silvex) inhibited the uptake of oxygen about the same extent (55 percent).

The effects of the sodium salts of the phenoxy compounds on oxygen uptake by bluegill liver mitochondria in the presence of alpha-ketoglutarate as substrate are summarized in Table 2. The sodium salts of the phenoxyacetic and phenoxypropionic acid did not alter oxygen uptake by bluegill liver mitochondria. The 2-CPA, 2,4-D and 2,4,5-T inhibited oxygen uptake by bluegill liver mitochondria, and the effects ranged from 58 percent (2,4-D) to 70 percent (2,4,5-T).

Phenoxypropionic acid did not alter oxygen uptake. The dichloro and trichloro substituted phenoxypropionic acid inhibited oxygen uptake 78 and 87 percent respectively.

The effects of the phenoxy compounds on the phosphate uptake by bluegill liver mitochondria are also summarized in Tables 1 and 2. In the presence of succinate, neither phenoxyacetic acid nor the phenoxypropionic acid altered phosphate uptake,

TABLE 2.—Effects of chloro phenoxy compounds on alpha-ketoglutarate oxidase.

		CONTROL		EXPERIMENTAL	
Derivative	g x 10^{-4} per ml Reaction medium	u10_2/hr/ mg N (Ave.)	umoles PO_4/hr/ mg N (Ave.) (1)	μl 0_2/hr/ mg N (Ave.)	μmoles PO_4/hr/ mg N (Ave.) (1)
Phenoxyacetic	1.5	153	32	151	14
# exp.		(4)	(4)	(4)	(4)
Range		(135-169)	(11-72)	(107-171)	(4-35)
2-chloro	1.9	168	40	71	20
# exp.		(4)	(4)	(4)	(4)
Range		(116-214)	(14-57)	(0-153)	(10-48)
4-chloro	3.4	168	40	58	32
# exp.		(4)	(4)	(4)	(4)
Range		(116-214)	(14-57)	(0-88)	(7-48)
Dichloro	3.7	164	22	68	22
# exp.		(4)	(5)	(5)	(5)
Range		(91-214)	(6-33)	(0-131)	(6-33)
Trichloro	2.7	172	40	47	22
# exp.		(3)	(3)	(4)	(4)
Range		(37-229)	(24-49)	(0-119)	(27-42)
Phenoxypropionic	1.7	153	32	156	18
# exp.		(4)	(3)	(4)	(4)
Range		(135-169)	(11-72)	(111-201)	(3-37)
Dichloro	2.4	152	40	32	9
# exp.		(3)	(3)	(3)	(3)
Range		(37-229)	(24-49)	(0-95	(8-15)
Trichloro	2.7	161	52	21	7
# exp.		(5)	(5)	(6)	(6)
Range		(37-229)	(24-91)	(0-56)	(5-19)
Phenoxybutyric dichloro	2.5	131	36	23	+63 (2)
# exp.		(4)	(4)	(4)	(4)
Range		(80-170)	(25-41)	(0-70	(32-81)

(1) Decrease in phosphate content of reaction media.

(2) Increase in phosphate content of reaction media.

but in the presence of alpha-ketoglutarate as substrate, both phenoxyacetic and phenoxypropionic acid appeared to uncouple the uptake of phosphate from the oxidation of substrate. All the chloro substituted phenoxy compounds used in this study did not appear to alter the uptake of phosphate by bluegill liver mitochondria in the presence of either substrate, as th eprimary effects of these chloro substituted phenoxy compounds appeared to be on oxygen metobolism.

The sodium salts of phenoxyacetic acid, 2-CPA,4-CPA,and 2,4,5-T did not alter the hydrolysis of ATP in the presence of manganese, magnesium, or calcium (Hiltibran, unpublished data). The sodium salt of 2,4-D (Hiltibran 1969a) did not alter the hydrolysis of ATP. The sodium salt of phenoxypropionic acid, dichlorprop, and silvex did not alter the hyrolysis of ATP in the presence of manganese, magnesium or calcium (Hiltibran, unpublished data). The sodium salt of 4,(2,4-dichlorophenoxy)butyric acid (2,4-DB) also, did not alter the hydrolysis of ATP in the presence of manganese, magnesium and calcium (Hiltibran, unpublished data).

In the presence of succinate, the chloro group at the 2 position (2-CPA) had a greater influence on oxygen uptake than the chloro group at the 4 position (4-CPA). The presence of a second chloro group at the 4 position appeared to reduce the strong influence of the chloro group at the 2 position, and the presence of a 3rd chloro group at the 5 position appeared to counteract the effect of the chloro group at 4 position and enhanced the effect of chloro at the 2 position, as indicated by the greater inhibition of oxygen uptake in the presence of 2,4,5-T.

In the presence of alpha-ketoglutarate as substrate in inhibition of oxygen uptake did not vary as much in the presence of the various chloro phenoxy compounds, however, the inhibition of oxygen uptake was more severe. This is in contrast to previous reports from this laboratory which have indicated that the succinate oxidase complex of the bluegill liver mitochondria has been affected to a greater extent than the alpha-ketoglutarate oxidase complex in the presence of 2,4-D (Hiltibran 1969b), DDT (Hiltibran 1970a) and metals (Hiltibran 1970b).

The primary purpose was to describe the effects of the chloro groups on the oxygen and phosphate metabolism of bluegill liver mitochondria, but it is possible to pursue further the relation of structure to function on a biological system. Considering the structures of 2,4-D and 2,4,5-T with dichlorprop and silvex, 2,4-D and 2,4,5-T contains an acetic acid analog whereas the dichlorprop and silvex contains propionic acid analog. The data suggests, therefore, the presence of an additional methylene group within the molecule must also exert some influence on the oxygen uptake by bluegill liver mitochondria.

The effect of the acid analog is further illustrated by the more severe inhibition of oxygen uptake in the presence of the sodium salt of 4(2,4-dichlorophenoxy)butyric acid (2,4-DB) in the presence of both substrates, Tables 1 and 2. These data indicate that the acid analog in the phenoxy compounds also has an influence on the oxygen and phosphate uptake by bluegill liver mitochondria. These observations are extended and the biochemical effects of the further modification of the phenoxy compounds by the introduction of esters or salts on the acid analog are discussed (Hiltibran 1972b). Whereas the chloro groups used in this study did not alter phosphate uptake, some chlorophenoxy compounds appear to alter phosphate metabolism (Hilti-

bran 1972b) and some chlorophenoxy compounds also have been found to alter the hydrolysis of ATP by bluegill liver mitochondria (Hiltibran 1972a).

These data extend the observations of Lotlikar, Remmert and Freed (1968) which suggested the position of the chloro group on the phenoxyacetic acid influenced oxidative phosphorylation by cabbage mitochondria (*Brassica oleracea*, var. *capitata* L.).

They reported that compounds with chloro groups at the 4 position inhibited oxygen uptake to a greater extent than the chloro group at the 2 position, and the chloro groups at the 2 and 4 positions were more effective than chloro groups at either the 2 or 4 positions.

In the present study the 2,4,5-T derivatives were more effective on oxygen uptake than were 2,4-D derivatives in the presence of both substrates, whereas silvex was more effective than the dichlorprop in the presence of alpha-ketoglutarate only. These data are in contrast to those presented by Lotlikar et al. (1968). The two trichloro derivatives used in their study were less effective than were the dichloro derivatives. Lotlikar et al. (1968) found that a chlorophenoxy compound containing the butyric acid analog was more effective on oxidative phosphorylation than the similar chlorophenoxy compound containing the acetic acid analog. However, the propionic acid analog was not used in their study. Switzer (1957) reported the 2,4,5-T was slightly more effective on oxidative phosphorylation by isolated soybean mitochondria than was 2,4-D.

These findings indicate a specific interaction of each chlorophenoxy compound with each bluegill liver enzyme complex. There also appears to be a correlation of these effects on oxidative phosphorylation and the toxicity of phenoxy compounds to small bluegills, as the phenoxy compounds which were more toxic to small bluegills than were other phenoxy compounds, altered either oxygen or phosphate uptake by bluegills to a greater extent than phenoxy derivatives, which were less toxic to small bluegills (Hiltibran 1969b, Hiltibran 1972b).

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