Biochemical Pharmacology, Vol. 23, pp. 1577–1582. Pergamon Press, 1974. Printed in Great Britain

OXIDATIVE PHOSPHORYLATION AND RESPIRATION BY LIVER MITOCHONDRIA FROM POLYCHLORINATED BIPHENYL-INTOXICATED RATS*

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(*Received* 9 *August* 1973; *accepted* 31 *October* 1973)

Abstract—Respiration and oxidative phosphorylation by intact hepatic mitochondria isolated from rats fed polychlorinated biphenyls (PCBs) were studied polarographically using glutamate and succinate as substrates. High levels of PCBs fed for 3 weeks resulted in an increase in respiration and in ADP/O ratios. However, when PCBs were fed to rats for 20 weeks at 100 ppm, there was no significant difference from control values. Gas chromatographic determination of PCB levels in mitochondrial fractions revealed 2–3 μg/mg of protein for treated rats in the acute study and about 0·3 μg/mg in the chronic study. Addition of PCBs *in vitro* to control rat liver mitochondria caused an inhibition of oxidative phosphorylation and respiration at or above a concentration of 50 μg/mg of mitochondrial protein. Since 2,4-dinitrophenol did not initiate uncontrolled respiration in PCB-inhibited rat liver mitochondria, and since inhibition occurred with both substrates, it appears that PCB inhibition *in vitro* occurs at a site along the respiratory chain.

POLYCHLORINATED biphenyls (PCBs) are mixtures of several chlorinated biphenyl molecules which have caused concern due to their wide dispersal and persistence in the environment and their tendency to be cumulative in food chains, thus affecting higher animals including man. In survey studies, a wide variety of samples from birds, fish and other wildlife have been found to be contaminated with PCBs.[1–4] These compounds have been discovered in human adipose tissue, [5] and a large scale contamination of rice bran oil in Japan produced widespread human poisoning.[6]

The biological effects of the PCBs have been well documented.[7] Norback and Allen [8] treated rats with chlorinated aromatic hydrocarbons and correlated hepatic morphological changes including proliferation of the endoplasmic reticulum and formation of concentric membrane arrays with a marked increase in hepatic enzyme activity. Other investigators have also reported an induction of microsomal drug-metabolizing enzymes[9] and an increase in mitochondrial δ-aminolevulinic acid synthetase (ALA)[10] in liver from PCB-intoxicated animals. On the contrary, Pardini[11] has demonstrated a marked inhibition of respiratory enzyme systems in heavy beef heart mitochondria (HBHM) exposed *in vitro* to numerous PCBs. He commented that the results further demonstrated the toxic nature of the PCBs; however, he cautioned that the effect remained to be verified *in vivo*.

* Supported in part by U. S. Public Health Service grants ES-00472 and RR-00167 from the National Institutes of Health. Primate Center Publication Number 13–033.

This study presents evidence that there is an induction *in vivo* by PCBs of hepatic mitochondral oxidative phosphorylation and respiration. Further, the data suggest that the quantity of PCBs accumulated in these organelles during PCB intoxication would be insufficient to cause inhibition based solely on studies *in vitro*.

MATERIALS AND METHODS

Animals and treatment. Male, Sprague–Dawley rats (ARS/S-D, Madison, Wis.), weighing 135 g, were randomly divided into groups and housed in an air-conditioned windowless room which was illuminated from 7:00 a.m. to 7:00 p.m. daily. Animals were fed *ad lib.* and had free access to water. Control rats received ground Purina Rat Chow, while test rats received a similar diet to which PCBs were added. In the acute study, rats received 1000 ppm of Aroclor 1248 or 1254 (Monsanto Co., St. Louis, Mo.) for 3 weeks. Throughout the 20-week chronic study, rats received 100 ppm of Aroclor 1248, 1254, or 1262. In all cases, there were no significant differences in weight gain or food consumption between PCB-fed or control animals.

Preparation of mitochondria. After 24 hr of food deprivation, rats were sacrificed by decapitation, exsanguinated, and their livers quickly removed. Intact hepatic mitochondria were isolated in STE medium consisting of 0·25 M sucrose, 10 mM Tris-HCl (pH 7·3), and 1 mM ethylenediaminetetra-acetate (EDTA) according to the procedures of Sordahl *et al.*[12] Liver was homogenized at 4° in 8 vol. of STE. The crude homogenate was then spun at 800 *g* for 10 min and mitochondria were isolated from the supernatant by centrifugation at 9000 *g* for 15 min. The mitochondrial pellet was washed twice and resuspended in enough STE to yield a suspension containing about 20 mg protein/ml. Mitochondrial protein was estimated by the method of Lowry *et al.*[13] using bovine serum albumin as the standard.

Respiration and oxidative phosphorylation. Oxygen utilization was measured polarographically by the method of Chance and Williams[14] using an oxygraph fitted with a Clark electrode (model K-ICC, Gilson Medical Electronics, Middleton, Wis.). Mitochondria at a concentration of 1·0 mg protein/ml were placed in an air-saturated reaction mixture consisting of 75 mM KCl, 50 mM Tris-HCl (pH 7·4), 10 mM potassium phosphate, 5 mM $MgCl_2$, 1 mM EDTA, and 10 mM glutamate or 10mM succinate plus 5 μM rotenone which was estimated to contain 230 nmoles oxygen/ml.[15] Incubations were conducted in a 1·5-ml, water-jacketed glass chamber maintained at 30°. The addition of 500 nmoles ADP stimulated the respiratory rate and will be referred to as state 3 following the terminology of Chance and Williams.[14] State 4 is ADP-limited and ADP/O ratios are calculated from the micromoles of ADP added divided by the microatoms of oxygen consumed during state 3 respiration.

Liver mitochondria from control rats were evaluated as described above prior to and after additions to the incubation mixture of from 1 to 250 μg/mg of mitochondrial protein of Aroclor 1248 as a concentrated ethanolic solution. After several min of incubation, 2,4-dinitrophenol (DNP) was added to the reaction at a concentration of 0·1 mM in order to induce uncoupled respiration.

All chemicals were reagent grade (Sigma Chemical Co., St. Louis, Mo.) and solutions were prepared using deionized, glass-distilled water.

Levels of PCBs in mitochondria. A portion of the mitochondrial pellets from control and PCB-fed rats was homogenized in hexane. This solvent was subsequently evaporated under dry nitrogen in the presence of anhydrous sodium sulfate in a 40° water bath. After concentration, clean-up of the samples was carried out in Silica gel-filled disposable pipettes by elution with a 1 : 1 benzene-hexane solution.[16] The levels of PCBs were quantitated in a Hewlett–Packard Research Chromatograph using a Pyrex glass column packed with 2% SE-30 coated on Gaschrom Q (80-100 mesh) and operated at 170° with an argon-methane mixture (95–5 per cent) as the carrier gas.

Data evaluation. Data were expressed as group mean values ± 1 S. D. Analysis of variance was used to determine over-all statistical significance and Fischer's LSD test was subsequently employed to determine differences between means.[17]

TABLE 1. RESPIRATION AND OXIDATIVE PHOSPHORYLATION BY LIVER MITOCHONDRIA FROM CONTROL AND HIGH LEVEL PCB-FED RATS*

Group	Substrate	Respiratory rate (nmoles O_2/mg protein/min) State 3	State 4	ADP/O
Control	Glutamate	42·3 ± 3·8	11·9 ± 1·0	2·59 ± 0·06
Aroclor 1248	Glutamate	61·6 ± 3·3	8·5 ± 0·7	2·93 ± 0·12
Aroclor 1254	Glutamate	68·9 ± 5·9	9·6 ± 0·9	2·92 ± 0·15
Control	Succinate	88·6 ± 3·2	22·8 ± 1·9	1·55 ± 0·09
Aroclor 1248	Succinate	118·1 ± 8·7	38·6 ± 3·6	1·86 ± 0·12
Aroclor 1254	Succinate	135·0 ± 9·9	43·1 ± 4·2	1·79 ± 0·08

* Data were derived from five rats in each group and expressed as mean values ± 1 S. D. Rats were fed ground chow to which 1000 ppm PCBs were added during a 3-week period prior to sacrifice. Aroclor group values are significantly different from controls ($P < 0·05$).

RESULTS

Respiration and oxidative phosphorylation by liver mitochondria from rats fed 1000 ppm PCBs for 3 weeks show significant differences from control values (Table 1). In state 3 respiration, there was approximately a 50 per cent increase in Aroclor groups with both substrates. In controlled respiration where ADP is limited (state 4), there was a slight decrease in PCB-fed rats with glutamate but greater than a 75 per cent increase over control mitochondria with succinate. ADP to oxygen ratios were significantly increased in liver mitochondria from Aroclor-treated animals.

The ingestion of low levels of PCBs (100 ppm) over an extended period (20 weeks) caused insignificant differences in respiration and oxidative phosphorylation by hepatic mitochondria from rats (Table 2). With both glutamate and succinate, there were no significant differences in mitochondrial function regardless of which Aroclor compound was ingested. The protein content of the mitochondrial fraction isolated from rat liver in both the acute and chronic studies did not differ significantly between control and Aroclor groups.

Chromatographic determinations of PCB levels in liver mitochondrial pellets from rats that had received 1000 ppm for 3 weeks revealed approximately 2–3 μg/mg of

TABLE 2. EFFECT OF CHRONIC INGESTION OF PCBs ON RESPIRATION AND OXIDATIVE PHOSPHORYLATION BY RAT LIVER MITOCHONDRIA*

Group	Substrate	Respiratory rate (nmoles O_2/mg protein/min) State 3	State 4	ADP/O
Control	Glutamate	38·7 ± 5·4	12·7 ± 1·0	2·61 ± 0·07
Aroclor 1248	Glutamate	35·2 ± 3·9	10·8 ± 1·5	2·65 ± 0·11
Aroclor 1254	Glutamate	37·6 ± 4·2	11·5 ± 0·6	2·57 ± 0·08
Aroclor 1262	Glutamate	39·2 ± 2·3	11·1 ± 0·7	2·48 ± 0·16
Control	Succinate	87·9 ± 10·9	22·2 ± 3·1	1·69 ± 0·13
Aroclor 1248	Succinate	96·2 ± 12·1	24·1 ± 2·9	1·71 ± 0·11
Aroclor 1254	Succinate	80·6 ± 9·3	25·9 ± 4·3	1·66 ± 0·09
Aroclor 1262	Succinate	99·1 ± 13·5	23·4 ± 3·6	1·72 ± 0·15

* Data were derived from five rats in each group and expressed as mean values ± 1 S. D. Rats were fed ground chow to which 100 ppm PCBs were added throughout a 20-week feeding period. Aroclor group values do not differ significantly from control values.

protein. There was about 0·3 μg/mg of mitochondrial protein in the pellets from the liver of rats chronically exposed to the Aroclor compounds.

Table 3 shows the effect *in vitro* of varied concentrations of Aroclor 1248 on respiration, DNP-uncoupled respiration, and the ADP/O ratio with glutamate and succinate as substrates. Control rat liver mitochondria were not affected until 50 μg of PCB/mg of protein was added to the reaction mixture. Then there was a 50 per cent decrease in state 3 respiration with both substrates and a similar decrease in state 4 respiration with glutamate. The inhibition of respiration was paralleled by an uncoupling of oxidative phosphorylation which prevented estimation of ADP/O ratios. A decrease in DNP-stimulated respiration occurred as the concentration of the PCB was increased.

TABLE 3. EFFECT OF AROCLOR 1248 *in vitro* ON RESPIRATION AND OXIDATIVE PHOSPHORYLATION OF GLUTAMATE (G) AND SUCCINATE (S) BY CONTROL RAT LIVER MITOCHONDRIA*

Aroclor 1248 (μg/mg protein)	Substrate	Respiratory rate (nmoles O_2/mg protein/min) State 3	State 4	DNP	ADP/O
None		45·2 ± 0·7	12·2 ± 0·4	46·4 ± 0·6	2·59 ± 0·5
1	G	46·0 ± 0·5	11·9 ± 0·3	46·8 ± 0·5	2·61 ± 0·4
10	G	38·7 ± 0·9	11·9 ± 0·6	44·1 ± 0·7	2·52 ± 0·6
50	G	18·9 ± 1·1	6·7 ± 0·8	5·9 ± 0·8	†
100	G	†	†	†	†
250	G	†	†	†	†
None	S	85·5 ± 0·9	23·6 ± 0·5	92·1 ± 0·8	1·65 ± 0·4
1	S	85·1 ± 0·7	23·1 ± 0·6	90·9 ± 0·7	1·66 ± 0·3
10	S	83·6 ± 1·3	22·7 ± 0·8	90·9 ± 1·5	1·63 ± 0·7
50	S	37·8 ± 2·4	21·3 ± 1·2	17·8 ± 1·4	†
100	S	†	10·1 ± 0·8	8·4 ± 0·6	†
250	S	†	†	†	†

* Data are expressed as mean values ± S.D. of triplicate determinations on mitochondria from each of four different control rat livers.

† Only minimal respiration or oxidative phosphorylation occurring at a rate too slow to be estimated.

DISCUSSION

The exposure *in vivo* of rats to PCBs does not have an inhibitory effect on hepatic mitochondrial enzyme systems and, in fact, may cause an induction of respiratory enzymes under selected circumstances. It has clearly been established that PCBs are responsible for the proliferation of hepatic endoplasmic reticulum and the induction of enzyme activities in PCB-treated animals.[8,18] In addition, Vos *et al.*[10] have demonstrated a large increase in mitochondrial ALA synthetase activity in the liver of birds orally dosed with Aroclor 1260. The fact that high-level short-term exposure of rats to PCBs caused a significant increase in the tightness of coupling and the efficiency of phosphorylation suggests that an induction of hepatic mitochondrial enzyme activity may have occurred in these animals. Since the PCB-exposed hepatocyte is physiologically quite active, the increase in respiration and oxidative phosphorylation may simply reflect the need for an increase in the number of high-energy bonds of ATP. The possibility that exposure *in vivo* to PCBs specifically induces hepatic mitochondrial enzyme systems remains to be clarified.

The apparent lack of effect of the liver mitochondria from rats chronically exposed to low levels of PCBs is somewhat difficult to explain. It must be kept in mind that the net over-all exposure of rats to PCBs was considerably greater in the acute study than in the chronic study. This explains the greater PCB level (5–10 times) found in the mitochondria of the former group and may also account for the results observed, in that the exposure to the mitochondria in the chronic study might have been insufficient to elicit the induction of enzyme systems. Furthermore, the status of these organelles was evaluated only at the end of a 20-week feeding period when the liver could have somewhat adapted to the presence of PCBs and perhaps progressively sequestered them away from mitochondria to another cytoplasmic organelle such as the endoplasmic reticulum.

Certainly, the ability to partition PCBs *in vitro* is lacking and the present results substantiate the work of Pardini[11] in demonstrating the inhibitory action of PCBs on isolated mitochondria. He manometrically evaluated HBHM NADH-oxidase and succinoxidase systems in the presence of over 500 μg PCB/mg of mitochondrial protein and concluded that they were inhibitory. We have titrated mitochondrial enzyme activity using Aroclor 1248 and have shown that at a concentraion of less than 50 μg/mg of protein there is little or no inhibitory effect on rat liver mitochondria. It must be emphasized that this level of PCB is considerably greater than that which was present in our experiments *in vivo*, and therefore, it might have been predicted that there would be no effect *in vivo* at all, much less a slight inducing one.

The site of interaction of the PCBs during inhibition of mitochondrial electron transport systems *in vitro* appears to be on the substrate side of cytochrome c as noted by Pardini.[11] Uncoupling by DNP did not increase respiratory rate, and therefore, either inhibition also occurs at the DNP site or more likely the respiratory chain is inhibited. The fact that state 4 and DNP-uncoupled respiration decreased with PCB addition and were finally completely inhibited with both glutamate and succinate as substrates further supports this contention.

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