Phospholipid synthesis and exchange between rat liver microsomes and mitochondria in the presence of benzo(a)pyrene

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Abstract Benzo(a)pyrene injection increased the phospholipid content in membranes of rat liver mitochondria and microsomes. There was a large relative increase of phosphatidylcholine, especially in microsomes, as compared with normal liver. The quantity of phosphatidylethanolamine seemed not be affected and the other phospholipid classes decreased. In vivo [U-^{14}C]glycerol incorporation into phospholipids was greater after benzo(a)pyrene injection, especially into phosphatidylcholine. Liver microsomes derived from rats injected with [U-^{14}C]glycerol or from liver slices incubated with the same precursor were incubated with unlabeled mitochondria in the presence of 105,000 *g* supernatant fraction; labeled microsomal phospholipids exchanged to a greater extent in the presence of benzo(a)pyrene, suggesting either a stimulation of activity of the exchange proteins, or a relative membrane disorganization facilitating the phospholipid transfer.—**Baraud, J., and A. Maurice.** Phospholipid synthesis and exchange between rat liver microsomes and mitochondria in the presence of benzo(a)pyrene. *J. Lipid Res.* 1980. **21:** 347–353.

Supplementary key word phospholipid exchange protein

Only a few workers have studied the direct action of carcinogenic chemicals on the membrane phospholipids of non-transformed cells. Rohrschneider, O'Brien, and Boutwell (1, 2) have shown the stimulation of phospholipid metabolism, especially phosphatidylcholine, in mouse skin after phorbol ester treatment. After p-dimethylaminobenzene injection into rats, the rate of phospholipid synthesis in microsomes is increased (3). 20-Methylcholanthrene may also cause an increased concentration of phosphatidylcholine in the microsomes of rat hepatocytes (4, 5). In previous work from our laboratory (6, 7), the effect of benzo(a)pyrene on phospholipids of mouse fibroblast cultures was demonstrated; it induced a marked increase of total phospholipids, especially phosphatidylcholine.

It has been shown (8) that normal liver mitochondria do not have the capacity to synthesize phospholipids except cardiolipin. However microsomes are able to synthesize all of their phospholipids and some mechanism must exist to transfer phospholipids from their site of synthesis to the mitochondrial membranes. The most likely mechanism involves soluble phospholipid exchange proteins (9, 10). Several of these proteins have been purified (11, 12) and analyzed (13–15).

In this report we examine the effects of benzo(a)pyrene on microsomal phospholipid biosynthesis and on the phospholipid exchange between microsomes and mitochondria.

MATERIALS AND METHODS

Chemicals

Benzo(a)pyrene was obtained from Fluka (Switzerland). L-[U-^{14}C]glycerol-3-phosphate, ammonium salt (170 mCi/mmol) and [U-^{14}C]glycerol (180 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. The Minimum Essential Medium (Eagle) for cell cultures (M.E.M.) was supplied by Grant Island Biological Company, New York.

Animals

Adult male Wistar rats, weighing 200–250 g, were given a standard diet a week before and throughout the experiments. One group of three animals received 20 mg benzo(a)pyrene dissolved in 1 ml of arachis oil by intraperitoneal injection; the control group received 1 ml of arachis oil alone. After 5 days, all animals received by intraperitoneal injection 25 μCi L-[U-^{14}C]-glycerol-3-phosphate or [U-^{14}C]glycerol in 1 ml M.E.M.; 1 hr later they were killed by decapitation, and the livers were removed and minced as quickly as possible.

Tissue phospholipids

After rinsing the liver fragments with ice-cold "buffer A" (0.25 M sucrose, 0.15 M KCl, 50 mM Tris, 1 mM

Abbreviation: PLP, phospholipid-P.

EDTA, pH 7.5), a 5% homogenate was made in the same medium in a Dounce's homogenizer. A known aliquot was removed for determination of proteins and of total PLP.

Preparation of subcellular fractions

A 20% homogenate was made in buffer A with 10 strokes of a "Polybroyeur" homogenizer. The homogenate was centrifuged at 800 *g* for 15 min at 4°C to sediment whole cells, nuclei and cell fragments. The supernatant was centrifuged for 5 min at 15,000 *g* (Sorvall RC2B, rotor SS 34) to sediment mitochondria. The pellet was washed twice with the same buffer and sedimented each time as before.

The 15,000 *g* supernatant fraction was centrifuged at 4°C for 20 min at 15,000 *g* to sediment light mitochondria, lysosomes, and heavy microsomes; the pellet was discarded. The supernatant was centrifuged at 105,000 *g* for 120 min (M.S.E. angle rotor 8 × 50) and the sediment, consisting of microsomes, was washed with 40 ml of 10 mM Tris buffer, pH 8.6, then with 40 ml of 1 mM Tris buffer, pH 8.6 in order to remove intravesicular proteins, according to Wallach and Kamat (16). The microsomes were centrifuged each time at 105,000 *g* for 60 min. They were finally resuspended in 1 mM Tris. A known aliquot was removed for determination of proteins and the remainder was used for study of phospholipids after a final centrifugation.

Phospholipid exchange between labeled microsomes and unlabeled mitochondria

Labeling of microsomes in vivo. Radioactive microsomes were obtained from rats injected intraperitoneally with [U-^{14}C]glycerol (25 μCi in 1 ml M.E.M.). Rats were killed 1 hr after the injection. Unlabeled mitochondria and an unlabeled 105,000 *g* supernatant fraction were obtained from untreated rats. Mitochondria were suspended in 100 ml of the 105,000 *g* supernatant. Forty μg benzo(a)pyrene in 1 ml of acetone was mixed with 50 ml of the mitochondrial suspension. The remaining 50 ml of suspension was mixed with 1 ml of acetone alone, as a control. Labeled microsomes were suspended in 10 ml of buffer A and 5 ml of this suspension was mixed with each 50 ml of mitochondrial suspension. Incubations were performed at 37°C for 1 hr. Then mitochondria and microsomes were isolated as described above.

Labeling in vitro. Labeled phospholipids were also obtained from liver tissue slices. Rat liver was cut with a Stadie-Riggs microtome, and slices (0.3 mm thick) were incubated in 20 ml of buffer A + 1 ml [U-^{14}C]-glycerol (25 μCi) for 60 min in a shaking bath at 37°C. After homogenization, mitochondria or microsomes were recovered by centrifugation; the remainder of the procedure was similar to that described above.

Protein determination

Protein was measured by the method of Lowry et al. (17) using serum albumin as standard.

Extraction and separation of phospholipids

Lipids were extracted from whole cells and mitochondrial and microsomal pellets, with chloroform–methanol 2:1 (v/v), and the extracts were purified according to Folch, Lees, and Sloane Stanley (18). A known aliquot was removed for determination of total phospholipid phosphorus according to the method of Napias (19). The remainder of the phospholipids was separated by two-dimensional chromatography on activated silica gel chromatography paper (Whatman SG-81, 10 × 12 cm), developed in chloroform–methanol–ammonia–water 330:115:15:4.5 (v/v) (first dimension) and chloroform–methanol–acetic acid–water 320:40:50:10 (v/v) (second dimension). Before chromatography the lower half of the paper was impregnated with 2.5% EDTA and dried at 100°C for 60 min, according to the method of Steiner and Lester (20). Each spot, detected by exposing the paper to iodine vapor, was cut out and eluted with methanol–chloroform 2:1 (v/v). The phospholipid was measured by determination of phosphorus, as above.

Measurement of radioactivity

Radioactivity was measured by liquid scintillation counting. The dried total phospholipids or the individual spots after paper chromatography were transferred directly to counting vials containing 10 ml of a solution of 4 g of Omnifluor per liter of toluene. The radioactivity of each phospholipid was expressed as a percentage of the total phospholipid radioactivity.

RESULTS AND DISCUSSION

Effect of benzo(a)pyrene on hepatocyte phospholipids

Three rats received benzo(a)pyrene and [U-^{14}C]-glycerol-3-phosphate as described in Materials and Methods. The phospholipid to protein ratio × 100 was 13.9 in hepatocytes from control animals, compared with 16.9 for the treated rats. The radioactivity incorporated into phospholipids was 339 and 414 cpm per μg PLP, respectively. Thus the incorporation of the radioactive precursor was increased as a result of benzo(a)pyrene treatment. A similar finding was noted (6, 7) with mouse fibroblast cultures: 80 μg of benzo(a)-

TABLE 1. Phospholipid composition of mitochondria and microsomes from rat hepatocytes: effect of benzo(a)pyrene

Phospholipids	Mitochondria		Microsomes	
	Control	+ Benzo(a)pyrene	Control	+ Benzo(a)pyrene
	Percent of total PLP[a]			
Cardiolipin	14.1 ± 0.3	5.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
Phosphatidylethanolamine	31.2 ± 0.5	30.0 ± 0.3	25.8 ± 0.2	25.6 ± 0.3
Phosphatidylcholine	43.2 ± 0.6	54.4 ± 0.8	53.1 ± 0.8	57.2 ± 0.5
Sphingomyelin	1.0 ± 0.1	0.8 ± 0.1	6.6 ± 0.2	6.1 ± 0.2
Phosphatidylinositol + phosphatidylserine	10.5 ± 0.5	9.3 ± 0.2	14.3 ± 0.3	11.0 ± 0.3
$\frac{\text{Phospholipid}}{\text{protein}} \times 100$	23.2 ± 0.5	26.8 ± 0.5	35.5 ± 0.7	42.8 ± 0.8

[a] The results are the means of four separate experiments (three animals per experiment). Rats were killed 5 days after 20 mg benzo(a)pyrene injection.

pyrene per liter of culture increased the phospholipid to protein ratio × 100 from 15.5 to 19.0.

Benzo(a)pyrene caused a redistribution of the various phospholipids. Phosphatidylcholine increased 15% but the phosphatidylethanolamine content was practically unchanged. This indicated that benzo(a)pyrene stimulated phosphatidylcholine biosynthesis, perhaps by an increase in activity of *S*-adenosylmethionine-phosphatidylethanolamine-methyl-transferase, transforming phosphatidylethanolamine to phosphatidylcholine, as Young, Powell and McMillan (21) observed with phenobarbitone.

Effect of benzo(a)pyrene on liver microsomal and mitochondrial phospholipids (Table 1)

Total phospholipids. The phospholipid to protein ratio × 100 was 23.2 ± 0.5 and 26.8 ± 0.6 in control and benzo(a)pyrene-treated liver mitochondria, respectively. This ratio was 35.5 ± 0.5 and 42.8 ± 0.6 in microsomes.

Phospholipid composition. In the first set of experiments, rats were killed 5 days after benzo(a)pyrene injection. These animals did not receive the radioactive precursor. Benzo(a)pyrene increased the mitochondrial and the microsomal phosphatidylcholine compared with total phospholipids, and it appears that this phospholipid was responsible for the total phospholipid increase. Mitochondrial cardiolipin decreased in each experiment, suggesting that benzo(a)pyrene inhibits its mitochondrial biosynthesis.

In the second set of experiments the rats received [U-^{14}C]glycerol (25 μCi) 1 hr before they were killed. The data of **Table 2** show that the incorporation of the labeled precursor increased in the presence of benzo(a)pyrene; the specific activity of microsomal phospholipids increased from 1705 to 2293 cpm per μg PLP. This important effect of benzo(a)pyrene was not surprising because most phospholipid synthesis occurs in microsomes. It is known that carcinogenic hydrocarbons are subsequently converted into dihydrodiols by the action of microsomal oxygenases and these dihydrodiols are more active than benzo(a)pyrene itself (22, 23).

This increased amount of labeled phospholipids as a result of benzo(a)pyrene was also found in mitochondria. The specific activity increased from 718 to 938 cpm per μg PLP. Phosphatidylethanolamine and phosphatidylcholine were highly labeled, but the radioactivity in cardiolipin was very slight. This result is in agreement with McMurray and Dawson (24). The increase of mitochondrial labeled phospholipids may have been due to the increased specific activity of microsomal phospholipids.

Effect of benzo(a)pyrene on the phospholipid exchange

It is known that phospholipids synthesized in microsomes are transferred to different membranes by specific carrier proteins (9, 10). These proteins are present in a 105,000 *g* supernatant fraction of rat liver and transfer phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol from microsomes to mitochondria (9, 10, 24–26). For verification of an eventual action of benzo(a)pyrene on the phospholipid transfer, we have studied the exchange between microsomes and mitochondria, in the absence of de novo biosynthesis.

Effect of time of incubation on the exchange

Mitochondrial phospholipids (40 μg P) were incubated at 37°C with microsomal phospholipids (60 μg P) (sp act 2210 cpm/μg PLP) in a 105,000 *g* supernatant, with 40 μg benzo(a)pyrene in 1 ml of acetone or with 1 ml of acetone alone, as control. The total

TABLE 2. Incorporation of [U-^{14}C]glycerol into mitochondrial and microsomal phospholipids from liver cells of rats treated or not with benzo(a)pyrene

		Mitochondria		Microsomes	
		Control	+ Benzo(a)pyrene	Control	+ Benzo(a)pyrene
Specific activity (cpm per μg PLP)		718 ± 52	938 ± 60	1705 ± 89	2293 ± 104
		Radioactivity of phospholipid fractions			
Cardiolipin	a	0.5 ± 0.2	0.3 ± 0.1	0.0	0.0
Phosphatidylethanolamine	a	28.3 ± 0.8	23.5 ± 0.7	20.7 ± 0.5	20.5 ± 0.5
	b	652	734	1368	1836
Phosphatidylcholine	a	65.5 ± 1.0	71.0 ± 1.1	74.7 ± 1.1	72.3 ± 0.8
	b	1090	1223	2399	2899
Sphingomyelin	a	0.6 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	2.7 ± 0.2
	b	4.3	11.7	2.8	10.1
Phosphatidylinositol + phosphatidylserine	a	5.1 ± 0.3	4.2 ± 0.2	3.5 ± 0.1	4.3 ± 0.2
	b	348	423	417	837

a: % of total radioactivity.
b: specific activity (cpm per μg PLP).
20 mg benzo(a)pyrene and 25 μCi of [U-^{14}C]glycerol were injected 5 days and 60 min, respectively, before the animals were killed. Control rats received only 25 μCi [U-^{14}C]glycerol. The data are the mean of three separate experiments (three animals per experiment).

volume was 60 ml. At different times of incubation, 10 ml were drawn, mitochondria were separated, phospholipids were extracted and their radioactivity was measured. The percentage PLP exchange was expressed as mitochondrial radioactivity (cpm) × 100/ microsomal radioactivity (cpm) before incubation. **Fig. 1** shows PLP exchanged as a function of time. After 90 min the exchange was 8.2 and 14.2%, respectively, in the absence or in the presence of benzo(a)pyrene. Fig. 1 represents the data of a single experiment. Other experiments with slightly different concentrations of mitochondria, microsomes, and supernatant gave other data, but the graphs were similar.

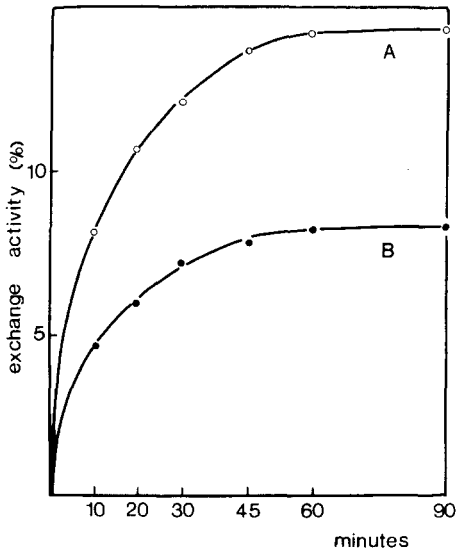

Fig. 1. Effect of time of incubation on the phospholipid exchange between labeled microsomes and unlabeled mitochondria incubated in a 105,000 *g* supernatant, with or without benzo(a)pyrene. The incubation mixture contained 40 μg mitochondrial PLP + 60 μg labeled microsomal PLP + 15 μg of 105,000 *g* supernatant PLP in a total volume of 60 ml. Total radioactivity in the microsomal phospholipids before incubation was 132,600 cpm (sp. act. 2210 cpm/μg PLP). Incubation was at 37°C. A: incubation in the presence of 40 μg benzo(a)pyrene in 1 ml acetone. B: incubation without benzo(a)pyrene (1 ml acetone alone). Exchange activity is expressed by dividing mitochondrial radioactivity × 100 by microsomal radioactivity before incubation.

Our data seem low as compared with 22.1% obtained by Wirtz and Zilversmit (25). However, in their experiments the exchange was performed with 41 μg of 105,000 *g* supernatant PLP in a total volume of 10 ml instead of 15 μg in a total volume of 60 ml for our experiment. Indeed, Wirtz and Zilversmit (9) have shown the fundamental influence of the exchange protein concentration.

Effect of benzo(a)pyrene on the exchange of various phospholipids

In vivo labeled microsomes were incubated with unlabeled mitochondria in an unlabeled 105,000 *g* supernatant fraction, with or without benzo(a)pyrene. After incubation for 1 hr at 37°C, the mitochondria and microsomes were separated and the specific activities of total phospholipids were determined. The radioactivity of each phospholipid fraction was measured after chromatography. An identical incubation was also carried out, as control, in buffer A + 10 mg bovine

TABLE 3. Exchange of phospholipids between in vivo labeled microsomes and unlabeled mitochondria, incubated in a 105,000 *g* supernatant, with or without benzo(a)pyrene

	Incubation without Benzo(a)pyrene		Incubation with Benzo(a)pyrene	
	Mitochondria	Microsomes	Mitochondria	Microsomes
Specific activity before incubation (cpm per μg PLP)		1143		1143
Specific activity after incubation in buffer (cpm per μg PLP)	36	1106	33	1099
Microsomal PLP exchanged %[a]	2.1		1.95	
Specific activity after incubation in supernatant (cpm per μg PLP)	117	980	210	877
Microsomal PLP exchanged %[a]	6.9		12.4	
Distribution of radioactivity in phospholipid fractions (%):				
Phosphatidylglycerol	0.5	0.4	0.5	0.7
Phosphatidylethanolamine	25.2	20.8	23.2	22.8
Phosphatidylcholine	65.7	63.7	70.1	60.6
Sphingomyelin	2.8	3.5	2.6	3.2
Phosphatidylinositol + Phosphatidylserine	5.8	11.6	3.6	12.7

[a] % PLP exchanged is: mitochondrial radioactivity (cpm) × 100/microsomal radioactivity (cpm) before incubation.

Microsomal phospholipids were labeled by 25 μCi [U-^{14}C]glycerol injected 1 hr before the rats were killed. Mitochondria and the 105,000 *g* supernatant were obtained from untreated rats.

31 μg mitochondrial PLP and 46 μg microsomal PLP were incubated at 37°C for 60 min in 55 ml buffer A or in 55 ml of 105,000 *g* supernatant (10 μg PLP) with 40 μg benzo(a)pyrene in 1 ml acetone, or with 1 ml acetone alone, as control.

serum albumin, without a 105,000 *g* supernatant (**Table 3**).

The percentage of microsomal PLP transferred into mitochondria was obtained by dividing total mitochondrial radioactivity (cpm) × 100 by microsomal radioactivity (cpm) before incubation.

After incubation without the 105,000 *g* supernatant, the phospholipid exchange between microsomes and mitochondria was very slight: about 2% of the labeled microsomal phospholipids were transferred into mitochondria and benzo(a)pyrene had no effect. This slight exchange may have been due to transfer by fusion or sticking of organelles.

After incubation with 105,000 *g* supernatant, the radioactivity of mitochondrial phospholipids was 3627 cpm (117 cpm/μg PLP), corresponding to 6.9% microsomal labeled phospholipids exchanged. In the presence of benzo(a)pyrene the radioactivity of mitochondrial phospholipids was increased to 6510 cpm (210 cpm/μg PLP) corresponding to 12.4% microsomal labeled phospholipids exchanged. For comparison of the transferred activity of each phospholipid, it was noted that phosphatidylcholine + phosphatidylethanolamine were 91–93% of total radioactivity; transfer of other phospholipids was slight and insignificant.

Two other sets of experiments were done according to a different procedure with in vitro labeled microsomes, as described in Materials and Methods, in order to obtain higher specific radioactivities. In the first set (**Table 4a**), benzo(a)pyrene increased the microsomal-labeled phospholipids transferred from 7.6 to 12.1%. In the second set (Table 4b) the exchange was increased from 9.2 to 15.8%.

Exchange reversibility

It is well known that exchange proteins transfer phospholipids between microsomes and mitochondria in both directions. Wirtz and Zilversmit (25) have shown that the amount of exchanged phospholipids from microsomes to mitochondria was identical to that exchanged from mitochondria to microsomes. We have performed studies to determine the effect of benzo(a)-pyrene in the two opposite directions of the exchange (**Table 5**).

Eighty μg of microsomal PLP, labeled in vitro (sp act 1530 cpm/μg PLP) was incubated with 55 μg unlabeled mitochondrial PLP in 55 ml of 105,000 *g* supernatant (12 μg PLP). After incubation for 60 min at 37°C, the mitochondria, microsomes, and supernatant were separated. The radioactivity of the mitochondrial phospholipids was 12,393 cpm, corresponding to 8.1 μg microsomal PLP exchanged, i.e., 10.1%. In the presence of 100 μg benzo(a)pyrene, the radioactivity of mitochondrial phospholipids was 20,502 cpm, cor-

TABLE 4. Exchange of phospholipids between in vitro labeled microsomes and unlabeled mitochondria, incubated in a 105,000 *g* supernatant, with or without benzo(a)pyrene

	Incubation without Benzo(a)pyrene		Incubation with Benzo(a)pyrene	
	Mitochondria	Microsomes	Mitochondria	Microsomes
Specific activity[a] (cpm per μg PLP)	175	1375	279	1235
Microsomal PLP exchanged %[c]	7.6		12.1	
Specific activity[b] (cpm per μg PLP)	257	1776	442	1527
Microsomal PLP exchanged %[c]	9.2		15.8	

[a] 50 μg mitochondrial PLP + 70 μg microsomal PLP (specific activity 1642 cpm/μg PLP) + 10 μg of 105,000 *g* supernatant PLP.

[b] 42 μg mitochondrial PLP + 55 μg microsomal PLP (specific activity 2130 cpm/μg PLP) + 13 μg of 105,000 *g* supernatant PLP.

[c] % PLP exchanged is: mitochondrial radioactivity (cpm) × 100/microsomal radioactivity (cpm) before incubation.

Microsomal phospholipids were labeled by incubation of rat liver slices in 20 ml buffer, pH 7.5, with 25 μCi [U-^{14}C]glycerol at 37°C for 60 min. Labeled microsomes were isolated and incubated with unlabeled mitochondria in 55 ml 105,000 *g* supernatant, with 40 μg benzo(a)pyrene in 1 ml acetone, or with 1 ml acetone alone, as control.

responding to 13.4 μg microsomal PLP exchanged, i.e., 16.75%.

On the other hand, 61 μg mitochondrial PLP, labeled in vitro (sp act 1038 cpm/μg PLP) was incubated with 89 μg unlabeled microsomal PLP in 55 ml of 105,000 *g* supernatant (12 μg PLP). After incubation (60 min at 37°C), the mitochondria, microsomes, and supernatant were separated by centrifugation. In the absence of benzo(a)pyrene, the radioactivity of microsomal phospholipids was 8304 cpm, corresponding to 8.0 μg mitochondrial PLP exchanged, i.e., 13.1%. In the presence of 100 μg benzo(a)pyrene, the radioactivity of microsomal phospholipids was 13494 cpm, corresponding to 13.0 μg mitochondrial PLP exchanged, i.e., 21.3%.

These experiments show that the same amount of phospholipid was exchanged in the two opposite directions: 8.1 and 8.0 μg PLP in the absence of benzo(a)pyrene, 13.4 and 13.0 μg PLP in its presence. On the other hand the specific activity of the supernatant phospholipids at the end of incubation was between those of mitochondria and microsomes when labeled microsomes were used, and was equal to those of microsomes when the mitochondria were labeled. These results are in agreement with Wirtz and Zilversmit (25). Furthermore our experiments showed an identical action of benzo(a)pyrene on the phospholipid exchange from microsomes to mitochondria and from mitochondria to microsomes; in both cases the exchange increased in the same proportion.

TABLE 5. Exchange of phospholipids between labeled microsomes and unlabeled mitochondria, or labeled mitochondria and unlabeled microsomes, incubated in 105,000 *g* supernatant, with or without benzo(a)pyrene

Experiment	Time of Incubation	Specific Activity			Radioactivity		
		Mitochondria	Microsomes	Supernatant	Mitochondria	Microsomes	Supernatant
	(min)		*(cpm/μg PLP)*			%	
Without benzo(a)pyrene[a]	0		1530		0	100	0
	60	225	1235	730	10.1	80.7	7.15
With 100 μg benzo(a)pyrene	0		1530		0	100	0
	60	373	1135	750	16.75	74.2	7.4
Without benzo(a)pyrene[b]	0	1038			100	0	0
	60	863	93	93	83.2	13.1	1.75
With 100 μg benzo(a)pyrene	0	1038			100	0	0
	60	761	151	155	73.3	21.3	2.9

[a] The incubation mixture contained 80 μg labeled microsomal PLP + 55 μg unlabeled mitochondrial PLP + 12 μg of 105,000 *g* supernatant PLP in a total volume of 55 ml. Total radioactivity in the microsomal PLP before incubation was 122,400 cpm.

[b] The incubation mixture contained 61 μg labeled mitochondrial PLP + 89 μg unlabeled microsomal PLP + 12 μg of 105,000 *g* supernatant PLP in a total volume of 55 ml. Total radioactivity in the mitochondrial PLP before incubation was 63,320 cpm.

Microsomal or mitochondrial phospholipids were labeled by incubation of rat liver slices in 10 ml buffer, pH 7.5, with 25 μCi [U-^{14}C]glycerol at 37°C for 60 min.

CONCLUSION

These experiments have shown that benzo(a)pyrene increases both microsomal phospholipid biosynthesis and phospholipid exchange between microsomes and mitochondria in both directions. Benzo(a)pyrene probably disorganizes more or less the phospholipid bilayer of microsomal and/or mitochondrial membranes, facilitating the phospholipid transfer from microsomes to carrier proteins and/or their insertion into mitochondria. It may not be out of the question to suggest that benzo(a)pyrene directly stimulates the activity of phospholipid exchange proteins.

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