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Fatty acids of liver mitochondrial and microsomal lipids in the rat exposed to phenothiazine derivatives

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DERIVATIVES of phenothiazine, e.g. chlorpromazine (2-chloro-10 [3-dimethylaminopropyl] phenothiazine) (CPZ) and prochlorperazine (2-chloro-10-[3-(-methyl-4-piperazinyl) -propyl] phenothiazine) (PCP) are major tranquilizers and can produce the symptoms of central nervous system depression in the rat. Although the specific site(s) of action of these drugs is not known, evidence by several workers suggests that they may directly affect the components of membranes.[1–3] Morphological changes have been described in liver and brain mitochondria of rats and monkeys following the administration of prochlorperazine.[4] The results indicated that a biochemical lesion may have been produced in the metabolism of the lipid components of these organelles. To examine the possibility that phospholipids are involved, gas–liquid chromatography was used to determine the effect of chlorpromazine and prochlorperazine *in vivo* on the proportions of fatty acids in the lipids of rat liver mitochondrial and microsomal fractions and on the fatty acids of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of the mitochondrial fraction.

The procedures employed were as follows: Male albino rats (Wistar strain, Woodlyn Farms, Guelph, Ontario, weighing 170–190 g), pre-adapted for 14 days to a purified diet containing 20% casein, 20% corn oil, 50% sucrose, 4% salt mixture, U.S.P. XIV, 1% vitamin mixture, and 5% alphacel, were injected intraperitoneally with a saline solution providing 5 mg/100 g body weight of chlorpromazine hydrochloride (Poulenc Limited, Montreal) or prochlorperazine ("Stemetil," Poulenc Limited, Montreal). At 4 and 24 hr after drug injection, groups of three animals were killed by decapitation and the livers rapidly removed. With a Dounce homogenizer, a 10% homogenate of liver tissue was prepared in 0·25 M sucrose (containing 10^{-3}M EDTA) from which the mitochondrial and microsomal fractions were separated by differential centrifugation.[5] The lipid was removed from each pelleted sub-cellular fraction by double extraction with water–methanol–chloroform (0·8 : 2 : 1, by vol.) and separation of the chloroform phase according to the method of Bligh and Dyer.[6] Fatty acid analyses of the total mitochondrial and microsomal lipid extract and of lecithin and phosphatidylethanolamine of the mitochondrial fraction were performed by gas–liquid chromatography.[7] Phospholipids were separated by thin-layer chromatography on silica gel H in a solvent system consisting of chloroform–methanol–glacial acetic acid–water (99 : 60 : 15 : 5, by vol.). One ml of butylated hydroxytoluene (BHT) in chloroform (9 mg/ml) was added as an antioxidant to the solvent to give a final concentration of 0·005%.[8] The phospholipid distribution in the liver mitochondrial fractions was estimated from the amount of lipid phosphorus in each phospholipid spot measured according to Bartlett[9] as modified by Parker and Peterson.[10]

The relative proportions (weight per cent) of major fatty acids of the liver mitochondrial and microsomal fractions from control and CPZ or PCP-treated rats were compared at 4 and 24 hr after drug injection (Table 1). In animals exposed to CPZ or PCP, the proportion of linoleic acid (18 : 2) was found to be significantly higher ($P = 0·05$) at 4 hr in the mitochondrial and microsomal fractions. Concurrently, the proportion of arachidonic acid in animals exposed to the drugs tended to decrease. These effects were no longer evident 24 hr after drug treatment. The differences in proportions of fatty

TABLE 1. MAJOR FATTY ACIDS OF LIVER MITOCHONDRIAL AND MICROSOMAL FRACTIONS FROM RATS GIVEN CHLORPROMAZINE (CPZ) OR PROCHLORPERAZINE (PCP)*

Fraction	Treatment		Fatty acid (wt. per cent)†					
			16:0‡	18:0	18:1	18:2	20:4	22:6
Mitochondria	Control	4 hr	16·0 ± 0·9	18·5 ± 0·5	7·3 ± 0·6	19·6 ± 0·3	25·2 ± 1·2	8·0 ± 0·8
	CPZ	4 hr	14·3 ± 0·5	18·7 ± 0·9	8·1 ± 0·6	23·9 ± 0·5§	23·1 ± 0·7	7·9 ± 0·7
	PCP	4 hr	15·9 ± 1·0	17·7 ± 0·3	8·3 ± 0·1	23·1 ± 0·6§	23·2 ± 0·4	7·6 ± 0·6
Mitochondria	Control	24 hr	16·7 ± 0·2	21·6 ± 0·5	5·4 ± 0·3	15·9 ± 0·1	27·8 ± 0·9	9·9 ± 0·8
	CPZ	24 hr	16·9 ± 0·5	21·1 ± 0·8	5·2 ± 0·1	16·6 ± 0·7	28·6 ± 0·5	8·6 ± 0·7
	PCP	24 hr	16·3 ± 0·6	21·7 ± 0·6	5·5 ± 0·2	16·8 ± 0·4	27·9 ± 0·7	9·5 ± 0·7
Microsomes	Control	4 hr	16·5 ± 0·2	21·3 ± 1·9	6·9 ± 0·7	18·2 ± 0·7	25·6 ± 0·6	7·0 ± 0·7
	CPZ	4 hr	16·2 ± 0·2	19·9 ± 0·2	8·5 ± 0·2	21·9 ± 0·5§	23·9 ± 0·6	6·5 ± 0·4
Microsomes	Control	24 hr	16·0 ± 0·6	23·0 ± 0·7	5·5 ± 0·1	14·3 ± 0·4	28·2 ± 0·5	8·6 ± 0·6
	CPZ	24 hr	18·0 ± 0·5	21·6 ± 0·6	5·8 ± 0·3	15·5 ± 1·2	27·7 ± 1·1	9·1 ± 0·5

* Samples were prepared at 4 and 24 hr after drug was given. Conditions of analyses were those described in the text. All values are expressed as the mean ± S.E. of three control and three drug-treated rats.

† Lipid extracts contained minor to trace amounts of 16:1, 17:0, 20:2, 20:3, 22:3 and 22:4.

‡ Number of carbon atoms: number of double bonds.

§ Significantly different from the control at $P = 0.05$.

TABLE 2. EFFECT OF CHLORPROMAZINE (CPZ) ON MAJOR FATTY ACIDS OF PHOSPHATIDYLCHOLINE (PC) AND PHOSPHATIDYLETHANOLAMINE (PE) OF THE LIVER MITOCHONDRIAL FRACTION*

Phospholipid	Treatment	Fatty acid (wt. per cent)†					
		16:0‡	18:0	18:1	18:2	20:4	22:6
PC	Control	19·7 ± 1·1	19·3 ± 0·2	5·9 ± 0·1	19·4 ± 1·0	23·9 ± 0·4	4·8 ± 0·3
	CPZ	18·5 ± 0·4	20·7 ± 1·1	6·0 ± 0·2	23·5 ± 0·5§	20·8 ± 0·4§	4·5 ± 1·0
PE	Control	14·5 ± 0·3	28·1 ± 0·8	5·7 ± 0·5	7·3 ± 0·2	27·7 ± 0·7	9·3 ± 1·1
	CPZ	13·9 ± 0·2	29·8 ± 0·9	5·1 ± 0·2	8·5 ± 0·5	27·5 ± 0·4	7·9 ± 1·4

* Mitochondrial fractions were prepared 4 hr after drug injection. Phospholipids were separated by thin-layer chromatography and the fatty acids analyzed as described in the text. All values are expressed as the mean ± S.E. of three control and three drug-treated rats.

† Lipid extracts contained minor to trace amounts of 16:1, 17:0, 20:2, 20:3, 22:4 and 22:5.

‡ Number of carbon atoms: number of double bonds.

§ Significantly different from the control at $P = 0{\cdot}05$.

acids seen at 4 hr in animals exposed to the drugs were apparently unrelated to a drop in body temperature since the effect was also observed in rats maintained at 32°,* a temperature known to prevent the hypothermia produced by exposure to phenothiazine derivatives.[11]

The proportions of major fatty acids in lipid extracts of the mitochondrial and microsomal fractions at 24 hr differed from that seen at 4 hr after drug injection because the animals were without food during the 24-hr test period. These changes were not unexpected, since it is known that fasting can affect the relative proportions of fatty acids in lipid extracts of rat liver tissue.[12,13] Within the group given CPZ (or PCP), the changes in the proportions of fatty acids (wt. per cent) observed between 4 and 24 hr closely paralleled the changes in the controls. Clearly, therefore, the effect of drug treatment on the proportion of linoleic acid was unrelated to food restriction. The changes in mitochondrial fatty acid composition observed at 4 hr were in the PC fraction, with no change in PE fatty acids (Table 2) or in the relative proportions of PC and PE which represented about 49 and 32 per cent, respectively, of the total lipid phosphorus in the mitochondrial fractions (Table 3).

TABLE 3. PHOSPHOLIPID COMPOSITION OF THE RAT LIVER MITOCHONDRIAL FRACTION*

Spot number	Mean recovery of phosphorus† (%)	Tentative identification
7 (everything above PE)	5·0 ± 0·3	Cardiolipin + phosphatidic acid
6	32·4 ± 1·4	Phosphatidylethanolamine
5	7·5 ± 0·4	Phosphatidylinositol
4	3·1 ± 0·3	Phosphatidylserine
3	48·6 ± 0·7	Phosphatidylcholine
2	2·8 ± 0·5	Sphingomyelin
1	2·8 ± 0·5	Lysophosphatidylcholine
0 (origin)	1·0 ± 0·2	Non-lipid phosphorus
Total	103·3 ± 2·3	

* Phospholipids were separated from extracts of mitochondrial lipid by thin-layer chromatography and analyzed for phosphorus content as described in the text. All values are expressed as the mean ± S.E. of three rats.

† Expressed as a percentage of the total phosphorus applied.

Variations in the fatty acid composition of membrane phospholipids may affect the properties of membranes.[14] The question remains, however, whether or not the differences in fatty acid composition seen here are related to changes in the properties of membranes. The significance of this question gains emphasis from the observation that the level of protein in rat liver mitochondria, expressed on a dry weight basis, appeared not to be affected by intraperitoneal administration of CPZ or PCP.* It is possible, therefore, that active involvement of phenothiazine derivatives at the level of the cell membrane might affect a conformational change in a membrane protein, the receptor, which then could initiate further events involving the lipids.[15]

Phenothiazine administration has been reported to alter temporarily the morphology of rat liver mitochondria.[4] It therefore seems probable that the transient changes in relative proportions of phospholipid fatty acids seen in rats exposed to phenothiazine derivatives are associated with the structural aspects of liver cell membranes.

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Effects of the carcinogen methylazoxymethanol acetate on protein synthesis and drug metabolism in rat livers*

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Methylazoxymethanol (MAM) acetate is a synthetic derivative of cycasin, a carcinogen derived from plants of the cycad family.[1,2] Cycasin, MAM and MAM acetate have been shown to induce tumors primarily in the liver, kidney, and intestine of rats after a single or small number of doses.[3–5] Zedeck *et al.*[5] reported that there was a rapid inhibition of DNA, RNA and protein synthesis which occurred in liver within 3 hr after giving MAM acetate. In addition, nucleolar damage was evident within 1 hr and preceded inhibition of nuclear RNA synthesis. Disaggregation of polysomes and depletion of rough endoplasmic reticulum have also been observed to occur in rat liver within a few hours after giving MAM.[6,7] The present study was undertaken to determine the effects of MAM acetate on various functions of the microsomal fraction of rat liver in order to further elucidate biochemical actions of the carcinogen which might be involved in the initial events leading to formation of neoplasia. The effects of MAM acetate were compared to those of dimethylnitrosamine (DMN), a chemically similar carcinogen. The agents were found to have very similar effects on protein synthesis and drug-metabolizing enzymes.

MAM acetate was purchased from Mann Research Laboratories, Inc., dimethylnitrosamine from Eastman Kodak Co., and L-leucine-4,5-^{3}H (38·5 c/m-mole) from New England Nuclear Corp. Male Sprague–Dawley rats (60–70 g) were obtained from Spartan Farms, Lansing, Mich. and allowed food and water *ad lib.*

All injection solutions were prepared in 0·9% NaCl. Injections were given intraperitoneally in a volume of 0·2 ml with the exception of pulse-doses of L-leucine-^{3}H, which were given by tail vein injection. Control animals were injected with saline solution. All animals were sacrificed by decapitation. For the drug metabolism studies, the animals were sacrificed between 8 and 9 a.m. In the case of polysome preparations, all animals were sacrificed between 1 and 2 p.m. to avoid possible diurnal variations in polysome patterns.[8]

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