Effect of Amitriptyline or Phenobarbital on the Activities of the Enzymes Involved in Rat Liver

Katsuji HOSHI* and Sumiko FUJINO

Department of Pharmacology, Hokkaido Institute of Pharmaceutical Sciences, 7–1 Katsuraoka-cho, Otaru 047–02, Japan. *Received June 6, 1990*

The effect of phenobarbital (PB) or amitriptyline (AMT) on phospholipid metabolism was studied in rat liver. Administration of PB or AMT caused a marked increase in the microsomal phosphatidylcholine (PC) content. In this context, the activities of glycerophosphate acyltransferase (GAT), phosphatidate cytidylyltransferase (PCT), phosphatidate phosphohydrolase (PPH) and choline phosphotransferase (CPT) in the liver were all increased by PB. In contrast, the activities of GAT and PCT were little affected by AMT, while those of PPH and CPT were significantly increased by AMT. These findings suggest that AMT or PB would increase hepatic microsomal PC synthesis through inducing PPH and then CPT.

Keywords amitriptyline; phenobarbital; phospholipid; phosphatidylcholine; glycerophosphate acyltransferase; phosphatidate cytidylyltransferase; phosphatidate phosphohydrolase; choline phosphotransferase

Introduction

Phosphatidylcholine (PC) is the most important phospholipids in mammalian tissues. Ishidate and Nakazawa[1] and Ilyas *et al.*[2] reported that phenobarbital (PB) caused a marked increase of phospholipid levels.

The biosynthesis of PC in the liver is regulated by several phospholipid biosynthesis enzymes. The primary precursor for the formation of diacylglycerol (DG) is phosphatidic acid (PA). PA is synthesized *via* 1-acylglycerol 3-phosphate by the stepwise acylation of *sn*-glycerol 3-phosphate.[3] PA lies at an important branchpoint in glycerolipid synthesis and provides two routes for further phospholipid metabolism. One is the pathway involved in the phosphorylation of PA to cytidine 5′-diphospho (CDP)-DG, which is subsequently converted into phosphatidylinositol (PI),[4] while the other is the pathway that PC is synthesized by the reaction of CDP-choline and DG *via* PA.[5–7]

Recently, we have demonstrated that amitriptyline (AMT) or PB caused a significant increase of hepatic drug-metabolizing enzyme activity.[8–10] Such a marked increase of hepatic drug-metabolizing activity by AMT is thought to influence phospholipid and related enzymatic activity.

The present study was therefore designed to investigate the effect of AMT or PB alone on phospholipid and its related enzymes in rat liver.

Experimental

Chemicals Glycerol 3-phosphate, disodium salt, L-[^{14}C(U)] (55 mCi/mmol), was purchased from American Radiolabeled Chemicals Inc., America. Cytidine 5′-diphospho [methyl-^{14}C] choline, ammonium salt (47 mCi/mmol) was purchased from Radiochemical Centre, Amersham, England. Other reagents were of analytical grade.

Treatment of Animals and Preparation of Subcellular Fractions Male Sprague Dawley rats weighing 170—185 g were used in all experiments. They were housed three per cage, fed Oriental MF laboratory chow, and had free access to water prior to sacrifice. Rats were orally given AMT in a single dose of 600 mg/kg. PB was injected intraperitoneally at a single dose of 80 mg/kg. The control animals received an equivalent volume of saline. Animals were sacrificed by decapitation 6, 12, 18 and 24 h after acute administration of the drug. The liver was removed immediately and homogenized in 5 vol. of 0.25 M sucrose containing 2.5 mM ethylenediaminetetraacetic acid (EDTA) and 50 mM Tris–HCl (pH 7.4) in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 9000 × *g* for 20 min in a Hitachi model 20 PR-5 centrifuge. The resulting supernatant was centrifuged at 105000 × *g* for 1 h in a Hitachi 65P-7 ultracentrifuge. The pellet was suspended in 8 ml of 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.4) and again centrifuged at 105000 × *g* for 1 h. The pellet was finally resuspended in 3 ml of 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.4) and used as a microsomal fraction.

Enzyme Assays Preparation of membrane-bound [^{14}C] phosphatidate was carried out by the method of Ide and Nakazawa.[4] The activities of GAT (acyl-CoA: *sn*-glycerol 3-phosphate *O*-acyltransferase; EC 2.3.1.15), PPH (phosphatidate phosphohydrolase; EC 3.1.3.4) and PCT (CTP: phosphatidate cytidylyltransferase; EC 2.7.7.41) were assayed by the method of Ide and Nakazawa.[4] CPT (choline phosphotransferase; EC 2.7.8.3) activity was assayed by the method of Sribney and Lyman.[11] Protein was determined according to the method of Lowry *et al.*[12]

Phospholipid Analysis of Microsomal Fraction Individual phospholipids (PC, PE and PI) were extracted by the method of Bligh and Dyer,[13] and aliquots were taken for lipid phosphorus determinations. Determination of phospholipids in rat microsomes was performed by gas chromatography–mass spectrometry according to the method of Hoshi *et al.*[14]

Results

Effects of AMT or PB on the Activities of Hepatic GAT, PCT, PPH and CPT and Microsomal Protein Content Figure 1 illustrates the time course of the administration of AMT or PB on the activities of GAT, PCT, PPH and CPT in rat liver. GAT activity increased by 232% when measured 12 h after PB-treatment, and subsequently returned nearly to control levels at 24 h. However, GAT activity by AMT remained unchanged. Moreover, at 12 and 18 h the PCT activity also increased to about 3.8- and 2.0-fold by PB, but that by AMT slightly decreased. On the other hand, a significant increase of PPH activity was observed 6 h after PB-treatment. The elevated enzyme activity due to PB-treatment reached peak level (about 3.0 times to control level) between 12 and 18 h and sustained it for 24 h. Similarly, AMT produced a steady sustained increase in PPH activity, which reached levels approximately 1.7- and 2.1-fold higher than the control levels at 12 and 18 h, respectively, and this activity was restored nearly to control level at 24 h. Furthermore, AMT caused a marked increase of CPT activity to 3.0 and 3.3 times, respectively, when measured 12 and 18 h after administration. On the other hand, at 12 and 18 h these activities were increased to about 3.0- and 2.5-fold by PB. This elevated enzyme activity was returned nearly to control levels at 24 h by AMT or PB. In this study, however, a significant increase of microsomal protein content by AMT or PB was not observed within 24 h (data not shown).

Effects of AMT or PB on Hepatic Phospholipid Contents Table I shows the content of hepatic microsomal PC after the administration of AMT or PB. The content of microsomal PC peaked at 12 h after the administration of AMT or PB. PC levels by AMT- or PB-treatments increased to

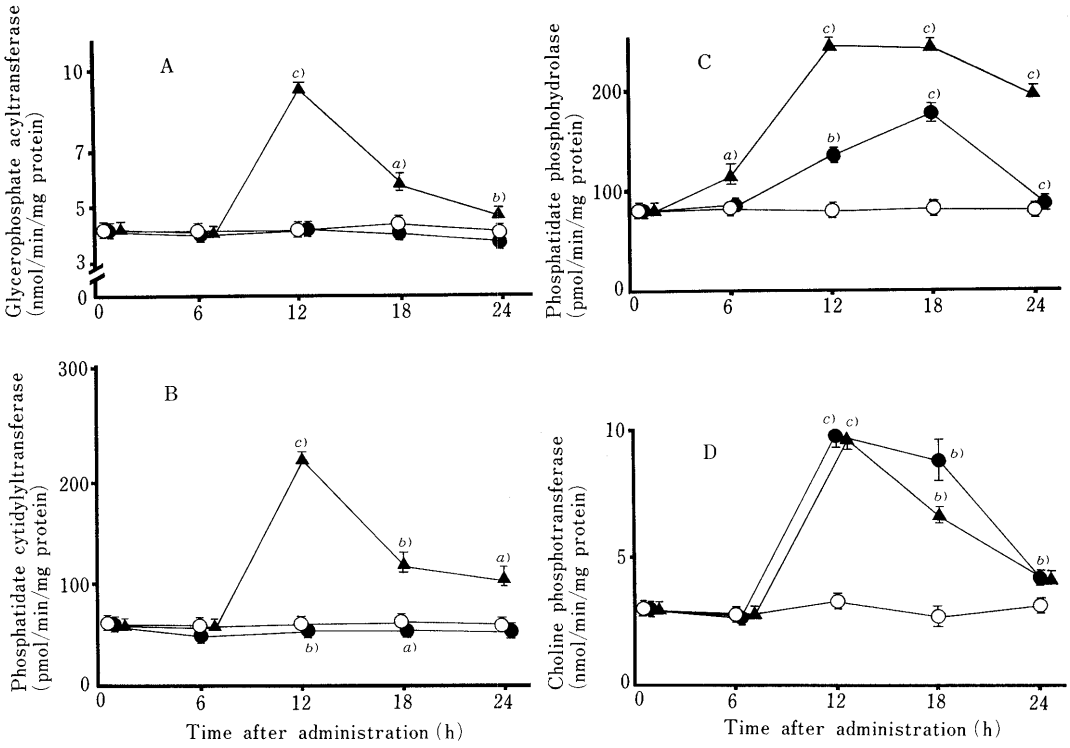

Fig. 1. Time Course of the Activities of GAT (A), PCT (B), PPH (C) and CPT (D) after the AMP or (PB)

Each point represents the mean±S.E.M. of 3—4 animals. ○, control; ●, AMP; ▲, PB. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ when compared with the control group.

TABLE I. Effect of a Single Administration of AMT or PB on Hepatic Microsomal PC Content in Rats

Time after the administration (h)	Control	AMP	PB
6	0.29±0.01	0.32±0.02	0.37±0.03[a)]
12	0.33±0.02	0.62±0.03[b)]	0.81±0.07[b)]
18	0.30±0.01	0.51±0.04[b)]	0.67±0.04[b)]
24	0.26±0.01	0.31±0.01[b)]	0.43±0.02[b)]

Doses of AMP and PB were 600 and 80 mg/kg, respectively. PC content is expressed as microsomes of phosphorus per mg microsomal protein. Each value represents the mean±S.E.M. of 6 animals. *a*) $p<0.05$, *b*) $p<0.001$ compared with the control group.

about 1.9- and 2.5-fold over the control level, and decreased (about 1.2—1.7 times to control levels) at 24 h. On the other hand, during the 6 to 24 h-period, the enhancement of PE contents by AMT or PB was less than that of PC contents. In addition, the content of PI did not change (data not shown).

Discussion

The present study demonstrates that a single administration of PB or AMT significantly increased hepatic microsomal PC and its related enzyme activity.

Ishidate and Nakazawa[1)] reported that PB significantly increased the rate of [^{32}P]incorporation into liver PC. Recently, Goldberg *et al.*[15)] showed that the enhanced rate of triacylglycerol synthesis in PB-treated rats was accompanied by an increase of the activity of DG acyltransferase, but not of PPH. However, in our result we observed a significant increase of PPH activity for 24 h and CPT activity also increased. In addition, PB resulted in a significant increase of PC content.

On the other hand, the activities of hepatic microsomal GAT and PCT in the present study increased to 232—376% of the control 12 h after the administration of PB. However, the contents of PI by PB remained unchanged. On the contrary, a significant increase of GAT and PCT activities by AMT was not observed within 24 h. Liver acyltransferase involving in *de novo* synthesis of PA from glycerophosphate is classified into two separate enzymes, GAT and 1-acylglycerophosphate acyltransferase.[3)] GAT activity in the present *in vivo* experiment was not changed by AMT when assayed with palmitoyl-CoA as the acyl-donor. The above fact is probably due to the enhanced level of PA synthesized *via* 1-acylglycerol 3-phosphate by the stepwise acylation of dihydroxyacetone phosphate and subsequent reduction of acyldihydroxyacetone phosphate. Furthermore, our results may indicate that the conversion of PA to PI in AMT-treated rats is little. In general, it is known that the biosynthesis of PC in the liver occurs mainly through two pathways: the Kennedy pathway (the most important route)[6)] and the methylation of PE.[16)] Finally, PC is synthesized by the reaction of CDP-choline and DG.[17,18)] In this connection, although most of the present evidence indicates that the rate of the reaction catalyzed by CPT is limited by the supply of CDP-choline, Tijburg *et al.*[19)] suggested that the amount of available DG can also determine the rate of PC biosynthesis. In this study, AMT caused a steady sustained increase in PPH activity. Moreover, the enhancement of CPT activity in the AMT-treated rats increased to the same degree as in the PB-treated rats. In addition, PC content was also increased by AMT.

In conclusion, our results suggest that the administration of AMT or PB would increase hepatic microsomal PC synthesis through inducing PPH and then CPT.

Acknowledgement We thank Mr. N. Senda for his excellent technical assistance.

References

1) K. Ishidate and Y. Nakazawa, *Biochem. Pharmacol.*, **25**, 1255 (1976).
2) M. S. Ilyas, F. A. Iglesia, and G. Feuer, *Toxicol. Appl. Pharmacol.*, **44**, 491 (1978).
3) R. Yada, H. Ide, and Y. Nakazawa, *Biochem. Pharmacol.*, **35**, 4083 (1986).
4) H. Ide and Y. Nakazawa, *J. Pharmacobio-Dyn.*, **3**, 612 (1980).
5) H. Ide and Y. Nakazawa, *Biochem. Pharmacol.*, **29**, 789 (1980).
6) E. P. Kennedy and S. B. Weiss, *J. Biol. Chem.*, **222**, 193 (1956).
7) R. H. Hjelmstad and R. M. Bell, *J. Biol. Chem.*, **262**, 3909 (1987).
8) K. Hoshi, N. Senda, T. Igarashi, T. Satoh, K. Ueno, and H. Kitagawa, *Res. Commun. Chem. Pathol. Pharmacol.*, **48**, 431 (1985).
9) K. Hoshi, N. Senda, and S. Fujino, *Res. Commun. Chem. Pathol. Pharmacol.*, **59**, 291 (1988).
10) K. Hoshi, N. Senda, and S. Fujino, *Jpn. J. Pharmacol.*, **50**, 289 (1989).
11) M. Sribney and E. M. Lyman, *Can. J. Biochem.*, **51**, 1479 (1973).
12) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
13) E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
14) K. Hoshi, N. Senda, and S. Fujino, *Res. Commun. Psychol, Psychiat., Behv.*, **15**, 41 (1990).
15) D. M. Goldberg, M. W. Roomi, A. Yu, and D. A. K. Roncari, *Biochem. J.*, **196**, 337 (1981).
16) D. E. Vance and N. D. Ridgway, *Prog. Lipid Res.*, **27**, 61 (1988).
17) P. Lim, R. Cornell, and D. E. Vance, *Biochem. Cell Biol.*, **64**, 692 (1986).
18) J. E. Vance and D. E. Vance, *J. Biol. Chem.*, **263**, 5898 (1988).
19) L. B. M. Tijburg, M. J. H. Geelen, and L. M. G. van Golde, *Biochem. Biophys. Acta*, **1004**, 1 (1989).