PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 143–146 (1981)

Effect of Alloxan Diabetes on Phosphatidylcholine Biosynthetic Enzymes[1] (41139)

DENNIS R. HOFFMAN, JUDY A. HANING, AND W. E. CORNATZER

Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota School of Medicine, Grand Forks, North Dakota 58202

Abstract. Phosphatidylethanolamine methyltransferase, phosphatidyldimethylethanolamine methyltransferase, and choline phosphotransferase enzymatic activities (nmole PC formed min/mg protein) have been determined in liver microsomes of alloxan diabetic rats. There was a significant reduction in the methylation pathway in the conversion of phosphatidylethanolamine to phosphatidyl choline as demonstrated in the low value of the phosphatidylethanolamine methyltransferase and phosphatidyldimethylethanolamine methyltransferase in those diabetic rats with blood glucose levels greater than 600 mg%. The reduction was 49 and 48% decrease over controls, respectively. There was a significant increase in the choline phosphotransferase in the diabetic rats. The increase was 92% over controls for the rats with blood glucose of 461 mg% and 55% over controls for the rats with blood glucose 946 mg%.

Phosphatidylcholine (PC) is the major phospholipid in plasma, nuclear, mitochondrial, and endoplasmic reticular (microsomes) membranes of all cells. Phosphatidylcholine represents 45% of total lipid phosphorus of mitochondria, 48.5% of total lipid phosphorus of microsomes (1, 2), and 37.4% of total lipid phosphorus of plasma membranes (3). Johnson and Cornatzer (1) report a significant decrease in whole liver microsomal phospholipid-P and total liver phospholipid-P per milligram in alloxan diabetic rats compared to controls. Corder and Kalkhoff (4) have demonstrated a similar significant decrease in total liver phospholipid-P and a decrease in [^{14}C]palmitate incorporation in liver phospholipids in diabetic rats. Karageosian *et al.* (5) observed a statistically significant reduction in the concentration of PC and phosphatidylethanolamine in liver of diabetic rats. Turakulov *et al.* (6) showed a similar reduction in the concentration of total liver PC. Phosphatidylcholine biosynthesis in liver microsomes is known to occur by two major different pathways. The Kennedy (7) pathway involves choline phosphotransferase which catalyzes the following reaction: cytidine diphosphocholine-1,2-diacylglycerol to form PC + CMP. The Bremer–Greenberg (8) pathway involves phosphatidylethanolamine methyltransferase which catalyzes the following reaction: phosphatidylethanolamine + *S*-adenosylmethionine, with progressive methylation to form PC. In view of these observations for a reduction of the PC concentration in the diabetic state, experiments were undertaken to measure the enzymatic activity of the two enzymes involved in hepatic biosynthesis of PC in alloxan diabetic rats.

Materials and Methods. Female albino rats of the Sprague–Dawley strain (150–190 g) were used in all experiments. Animals fasted 24 hr were made diabetic by injecting intraperitoneally 145 mg/kg body wt of alloxan monohydrate (9). After 9–12 days, animals with blood sugar levels above 360 mg/100 ml were selected for experimentation. The diabetic and control rats were killed by decapitation, the livers were removed, rinsed with cold water, blotted, and homogenized in ice-cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The microsomal fraction was isolated by differential centrifugation and protein was determined as previously described (10).

Choline phosphotransferase assay. The assay of the reaction catalyzed by the en-

[1] Supported by a grant from the American Diabetes Association, North Dakota Affiliate, Inc., and the Science and Education Administration of the U.S. Department of Agriculture, 5901-0410-8-0115, from the Competitive Research Grant Office.

0037-9727/81/060143-04$01.00/0

zyme CDP-choline:1,2-diacylglycerol choline phosphotransferase (EC 2.7.8.2) was done by the Kennedy and Weis method (7). The materials used were cytidine diphosphate-[1,2-^{14}C]choline (ICN Tracerlab Chemical and Isotope Division, Irvine, Calif.) and Tween 20 (Sigma Chemical Co., St. Louis, Mo.). Diacylglycerol was prepared from egg lecithin by the Gurr, Brindley, and Hübscher method (11) and purified by the Barron and Hannahan chromatographic method (12). Each reaction mixture contained 60 μmole 1,2-diacylglycerol emulsified in 0.1 ml of 1% Tween 20, 10 μmole $MgCl_2$, 0.5 μmole CDP-[1,2-^{14}C]-choline (sp act, 4×10^5 cpm/μmole), and 10 mg microsomal protein. The final volume of the reaction mixture was 1.3 ml. The reaction time was 6 min. The reaction was terminated by the addition of 3.0 ml 95% ethanol and the product was isolated by repeated extraction with ethanol and chloroform (7).

Phosphatidyldimethylethanolamine methyltransferase assay. The assay of the last step in the methylation of phosphatidylethanolamine to PC, catalyzed by phosphatidyldimethylethanolamine methyltransferase, was performed using the Rehbinder and Greenberg method (13). The reaction mixture contained 1.75 m*M* egg phosphatidyldimethylethanolamine (Avanti Polar Lipids, Birmingham, Ala.) 6.3 m*M* sodium deoxycholate, 0.3 *M* Tris–HCl buffer, pH 8.6, 0.35 m*M* *S*-adenosyl-L-[methyl-^{14}C]-methionine (0.1 mCi/mmole, New England Nuclear), and microsomes (1–2 mg) in a final volume of 1.15 ml. The assay was initiated by the addition of microsomes. Reaction time was 15 min at 37°. The reaction was terminated with 0.10 ml HCl and the product was isolated by the method of Bligh and Dyer (14).

Phosphatidylethanolamine methyltransferase assay. The rate-limiting step in the methylation pathway was measured by a modified method of Bremer and Greenberg (15). The reaction mixture contained 2 mg egg PC (Sigma Chemical Co.), 0.9 m*M* sodium deoxycholate, 0.3 *M* Tris–HCl buffer, pH 8.6, 0.2 m*M* *S*-adenosyl-L-[methyl-^{14}C]methionine (0.1 mCi/mmole, New England Nuclear), and microsomes (1–2 mg protein) in a final volume of 1.4 ml. The assay was initiated by adding microsomes, reaction time was 15 min at 37°, and the reaction was terminated with 0.15 ml of HCl. The ^{14}C-labeled reaction product, PC, was extracted according to the Bligh and Dyer procedure (14).

Results and Discussion. The enzymatic activities of choline phosphotransferase, phosphatidyldimethylethanolamine methyltransferase and phosphatidylethanolamine methyltransferase were linear with time and concentration of enzyme for the controls and diabetic animals as previously reported (16).

The effect of alloxan diabetes on the specific activities (nmole phosphatidylcholine formed/min/mg protein) of these enzymes in liver microsomes is given in Table I. There is a significant reduction in the methylation pathway in the conversion of phosphatidylethanolamine to PC as demonstrated in the low values of the phosphatidylethanolamine methyltransferase and phosphatidyldimethylethanolamine methyltransferase in diabetic rats with blood glucose levels greater than 600 mg%. There is a significant increase in the choline phosphotransferase in the diabetic rats. Young and Lynen (17) reported a similar increase in the specific activity of the choline phosphotransferase in ketotic diabetic rats; however, the blood sugar data were not given. A number of investigators have demonstrated that the "pool size" or total liver phospholipids decrease in diabetes (1, 4). The major phospholipid, PC, found in liver cellular membranes has been shown to decrease in diabetes (1, 5, 6). Johnson and Cornatzer (1) demonstrated this decrease in liver microsomes. It would be anticipated that this decrease in the pool size of PC in liver microsomes would be reflected in the biosynthetic pathway of PC synthesis. The data of Table I demonstrate a significant decrease in the specific activities of both phosphatidylethanolamine methyltransferase and phosphatidyldimethylethanolamine methylation in liver microsomes of diabetic rats with blood sugar greater than 600 mg%. This decrease in the nanomoles of phosphatidylcholine formed/min/mg protein produced by this methylating pathway could reflect the decreased concentration of PC

TABLE I. EFFECT OF ALLOXAN DIABETES ON PHOSPHATIDYLETHANOLAMINE METHYLTRANSFERASE, PHOSPHATIDYLDIMETHYLETHANOLAMINE METHYLTRANSFERASE, AND CHOLINE PHOSPHOTRANSFERASE ACTIVITIES IN LIVER MICROSOMES FROM FEMALE RAT

	No. of animals	Blood glucose (mg%)	Specific activity (nmole phosphatidyl choline formed/min/mg protein)		
			Phosphatidyl-ethanolamine methyltransferase	Phosphatidyl-dimethylethanolamine methyltransferase	Choline phosphotransferase
Control	6	106 ± 18[a]	1.47 ± 0.16	24.11 ± 3.69	3.33 ± 0.85
Diabetic	8	461 ± 139*	1.66 ± 0.31	26.97 ± 3.04	6.40 ± 1.60*
	5	946 ± 152*	0.75 ± 0.04*	12.53 ± 3.09*	5.18 ± 2.29**

[a] Values are standard deviations. The test of significance was applied between controls and diabetics.
* $P < 0.001$.
** $P < 0.01$.

observed in diabetic rats (1, 5, 6). The methylation pathway of the conversion of phosphatidylethanolamine to PC has been estimated to contribute an average of 28% of the total PC synthesized in rat liver (18). Wise and Elwyn (19) have estimated that the methylation pathway for the conversion of phosphatidylethanolamine to PC provides 13 μmole choline/day/g of liver or equivalent to the normal dietary intake of choline/day for the rat. Phosphatidylethanolamine, the substrate for the phosphatidylethanolamine methyltransferase occurs in high concentration in liver microsomes (1). The other synthetic pathway for PC biosynthesis is by choline phosphotransferase. The activity of the choline phosphotransferase in liver microsomes is directly dependent upon the level of dietary methionine and choline (10). The increased activity of choline phosphotransferase observed in diabetic rats may be due to changes in some other cellular compounds, such as cyclic AMP (20), which is known to influence the activity of this enzyme. The observed decreased synthetic pathway of PC biosynthesis by methylation of phosphatidylethanolamine in diabetic rats must be larger than the concomitant increase choline phosphotransferase pathway to result in the decreased total PC that has been observed in membranes of diabetic animals (1, 5, 6). What is regulating these two biosynthetic pathways in diabetes is not known. This decrease in the methylating pathway that is observed in diabetic rats could change the composition of phospholipids and properties of the cellular membranes since this pathway provides phosphatidylcholines with more polyunsaturated fatty acids than the CDP-choline pathway (21, 22).

1. Johnson J. D., and Cornatzer, W. E., Proc. Soc. Exp. Biol. Med. **131**, 474 (1969).
2. Tsao, Shuang-Shine, and Cornatzer, W. E., Lipids **2**, 41 (1967); Miller, J. E., and Cornatzer, W. E., Lipids **4**, 19 (1969).
3. Pfleger, R. C., Anderson, N. G., and Snyder, F., Biochemistry **7**, 2826 (1968).
4. Corder, C. N., and Kalkhoff, R. K., J. Lab. Clin. Med. **73**, 551 (1969).
5. Karageosin, C. G., Amirkhanian, H., and Amirkhanian, L. T., Voprosy Biokhimii Mozga **7**, 150 (1972).
6. Turakulov, Y. K., Saator, T. S., Isaev, E. I., and Sadykov, S. S., Problemy Endokrinologgi (Mosk.) **25**, 54 (1979).
7. Kennedy, E. P., and Weiss, S. B., J. Biol. Chem. **222**, 193 (1956).
8. Bremer, J. and Greenberg, D. M., Biochim. Biophys. Acta **37**, 173 (1960).
9. Sharma, C., Manjeshwar, R., and Weinhouse, S., J. Biol. Chem. **238**, 3840 (1963).
10. Hoffman, D. R., Uthus, E. O., and Cornatzer, W. E., Lipids **15**, 439 (1980).
11. Gurr, M. T., Brindley, D. N., and Hübscher, G., Biochim. Biophys. Acta **98**, 486 (1965).
12. Barron, E. J., and Hannahan, D. J., J. Biol. Chem. **231**, 493 (1958).
13. Rehbinder, D. and Greenberg, D. M., Arch. Biochem. Biophys. **109**, 118 (1965).
14. Bligh, E. G., and Dyer, W. J., Canad. J. Biochem. Physiol. **37**, 911 (1959).
15. Bremer, J. and Greenberg, D. M., Biochim. Biophys. Acta **37**, 173 (1960).
16. Skurdal, D. N., and Cornatzer, W. E., Int. J. Biochem. **6**, 579 (1975).

17. Young, D. L., and Lynen, F., J. Biol. Chem. **244**, 377 (1964).
18. Bjørnstad, P., and Bremer, J., J. Lipid Res. **7**, 38 (1966).
19. Wise, E. M., and Elwyn, D., J. Biol. Chem. **240**, 1537 (1965).
20. Niles, R. M., and Makarski, J. S., J. Biol. Chem. **254**, 4324 (1979).
21. Rytter, D., Miller, J. E., and Cornatzer, W. E., Biochim. Biophys. Acta **152**, 418 (1968).
22. Miller, J. E., and Cornatzer, W. E., Lipids **4**, 19 (1969).

Received August 1, 1980. P.S.E.B.M. 1981, Vol. 167.