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INFLUENCE OF BILE ACIDS ON THE SOLUBLE PHOSPHATIDIC ACID PHOSPHATASE IN RAT LIVER

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SUMMARY

Biliary drainage was found to increase soluble phosphatidic acid phosphatase activity in rat liver about two-fold. Treatment with cholestyramine increased the same activity with about 70%. Treatment with 1% cholic acid or chenodeoxycholic acid decreased the activity 76% and 93%, respectively.

It is suggested that the enterohepatic circulation of bile acids might be of some regulatory importance for biosynthesis of triglycerides in the liver. The findings are discussed in relation to previous clinical observations that changes in bile acid metabolism affect plasma triglyceride turnover.

Several lines of evidence suggest that the metabolism of plasma triglycerides is related to the metabolism of bile acids in man. Thus, patients with endogenous hypertriglyceridemia often exhibit an increased production of bile acids (1), and this appears to be linked to the presence of an enhanced synthesis of plasma triglycerides (2). Furthermore, induction of an enhanced bile acid formation with cholestyramine treatment or biliary drainage is associated with an increased production rate of triglycerides (3-5). Finally, suppression of bile acid synthesis with chenodeoxycholic acid, and in some patients also with cholic acid, results in a reduction of plasma triglyceride synthesis (3,6,7).

The nature of this relationship between production of bile acids and biosynthesis of triglycerides is unknown. Bile acid biosynthesis is mainly regulated by a negative feed-back mechanism, by which bile acids reabsorbed from the intestine inhibit the cholesterol 7α-hydroxylase, which is the rate-limiting

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enzyme in the over-all biosynthesis of bile acids (8). It was considered to be of interest to study the possibility that also the phosphatidic acid phosphatase (EC 3.1.3.4), which is suggested to be the rate-limiting enzyme in the synthesis of triglycerides in the liver (9) is affected by the flux of bile acids. In the present work, the effects of biliary drainage, cholestyramine feeding and feeding with bile acids on hepatic phosphatidic acid phosphatase activity have been studied in the rat.

EXPERIMENTAL PROCEDURE

Animal Treatments. Male rats of the Sprague-Dawley strain were used. In view of the recent finding that phosphatidic acid phosphatase activity is subject to a diurnal variation with a maximum in the dark period (10), rats with a reversed diurnal rhythm were used. The rats were starved over-night prior to sacrifice and assay of hepatic phosphatidic acid phosphatase activity. In some experiments the rats were fed a diet containing 5% cholestyramine (Questran, Bristol, Sweden) for 10 days. The drug also contained sucrose which was mixed in the control diet in the same concentration as in the cholestyramine diet (corresponding to 4% sucrose in total diet). In some experiments the rats were fed 1% cholic acid or 1% chenodeoxycholic acid in the diet for 10 days. During this time their body weight increased with 20%, with no significant difference between the different groups of animals.

Preparation of Soluble Phosphatidic Acid Phophatase. Liver homogenates (20%, w/v) were prepared in 50 mM Tris-Cl buffer, pH 7.4. A cytosolic fraction was prepared by centrifugation at 20,000 x g for 15 min and recentrifugation of the supernatant at 100,000 x g for 1 h. The phosphatidic acid phosphatase activity was then precipitated by ammonium sulfate, essentially as described by Lamb and Fallon (11). The cytosol was treated with 0.23 g ammonium sulfate per ml solution for 10 min at 4° C. The mixture was centrifuged at 10,000 x g for 10 min and the precipitate was washed gently with 50 mM Tris-Cl buffer containing 0.23 M sucrose. The precipitate was dissolved in 0.3 M sucrose and dialyzed over-night against 0.3 M sucrose containing 0.5 mM DTT.

Preparation of Membrane Bound ^{14}C-Labeled Phosphatidic Acid. Microsomal bound phosphatidic acid was prepared as described by Lamb and Fallon by incubation of 1-^{14}C-labeled stearate (obtained from Radiochemical Centre, Amersham, England) with rat liver microsomes in the presence of ATP, CoA, sn-glycerol-3-phosphate, albumin, and NaF (11). After incubation, the microsomes were removed by centrifugation at 100,000 x g for 1 h and washed once by resuspension of the pellet in 0.3M sucrose solution and recentrifugation at 100,000 x g. The surface of the microsomal pellet was gently washed twice with a small volume of 0.3 M sucrose, prior to suspension in 0.3 M sucrose to give a solution containing about 2 mg protein per ml. The microsomal suspension was then frozen and stored at -20°C prior to use. Before incubation, the thawed suspension was heated at 85°C for 5 min to remove intrinsic microsomal activity.

More than 95% of the radioactivity in the final preparation was obtained in the phosphatidic acid fraction and less than 0.5% in the diglyceride fraction as judged by thin-layer chromatography of an extract of the microsomal suspension. Assuming that no significant dilution had occurred with endogenous phsophatidic

acid, the specific radioactivity of the membrane bound phosphatidate was 4.2×10^3 c.p.m. per nmol.

Incubation Procedure. The incubation mixture contained phsophatidic acid phosphatase (30-100 ug of the above dialyzed protein), microsomal bound phosphatidate (39 nmol, 0.18×10^6 c.p.m.), Tris-maleate, 60 umol, pH 6.9, $MgCl_2$, 0.8 umol, in a total volume of 0.8 ml. The incubations were performed at 37°C for 15 min and were terminated by addition of 4 ml of chloroform - methanol (2;1, v/v).

Analysis of Incubation Mixture. The chloroform phase obtained in the extraction of the incubation mixture with 4 ml chloroform-methanol (2:1, v/v) was evaporated under nitrogen and the residue subjected to thin-layer chromatography using diisopropylene ether - acetic acid (24:1, v/v) as solvent. The solvent was allowed to move only 10 cm. The chromatoplate was then dried in air for 30 min and immediately subjected to a second chromatography, using hexane-diethyl ether-acetic acid (80:20:1, v/v/v) as solvent. The conversion of labeled phosphatidic acid into diglyceride was measured by scanning of the chromatoplates with a radioscanner (Berthold, Wildbad, West Germany).

The conversion was expressed as nmol converted per mg protein per min. Protein was determined according to the Lowry procedure (12).

RESULTS

In accordance with previous work (11), use of membrane bound phosphatidic acid was found to give more reproducible results than use of free phosphatidic acid. Precipitation of phosphatidic acid phosphatase with ammonium sulfate was found to be necessary. Thus, assay of the crude cytosolic fraction gave less reproducible results.

Under the conditions employed, the conversion was linear with time as well as with amount of enzyme, and this linearity occurred both under basal conditions and in a stimulated state. The conversion increased with increasing amounts of phosphatidic acid added up to about 40 nmol where a plateau was reached . Increasing the amount of added phosphatidate over 60 nmol, however, further increased the activity. Several experiments with different preparations of phosphatidic acid phosphatase and microsomal bound phosphatidate gave similar results. Thus it was not possible to obtain a true substrate saturation, and in all experiments about 40 nmoles of membrane bound phosphatidic acid was used as substrate.

Table I.

Effect of biliary drainage and feeding of cholestyramine and bile acids on phosphatidic acid phosphohydrolase activity.

The rats were treated with 5 % cholestyramine or 1 % cholic acid or 1 % chenodeoxycholic acid in diet for 10 days. Hepatic phosphatidic acid phosphatase activity was assayed as described in Experimental Procedures. In each set of experiments, groups of six rats were used and the results given are expressed as mean ± S.E.M.

	Phosphatidic acid phosphatase activity
	nmol/mg protein/min
Exp. I	
Control rats (sham operated)	1.83 ± 0.11
Bile fistula rats	4.38 ± 0.67
Exp II	
Control rats	1.91 ± 0.24
Cholestyramine treated rats	3.30 ± 0.30
Exp. III	
Control rats	1.35 ± 0.40
Cholic acid treated rats	0.33 ± 0.09
Chenodeoxycholic acid treated rats	0.10 ± 0.03

Table I summarizes the results of the different treatments on phosphatidic acid phosphatase activity. A biliary fistula increased the activity with about 140% ($p < 0.001$, Student's t-test), whereas treatment with cholestyramine increased the activity with about 70% ($p < 0.05$). Treatment with cholic acid decreased the activity with about 76% ($p < 0.05$), whereas treatment with chenodeoxycholic acid decreased the activity with more than 90% ($p < 0.02$). The difference between the effect of cholic acid and chenodexoycholic acid was statistically significant ($p < 0.05$).

DISCUSSION

Several lines of evidence suggest that the soluble hepatic phosphatidic acid phosphatase is the rate controlling enzyme in the biosynthesis of triglycerides in

the liver (9). The activity of this enzyme is known to change within a few hours after an alteration in the physiological state so as to parallel the observed rate of triacylglycerol synthesis,or the capacity of the liver to synthesize triacylglycerols. Other enzymes in the pathway do not change their activity at all, or the changes are smaller than those observed for the phosphatase (9).Also the microsomal fraction of a liver homogenate contains phosphatidic acid phosphatase. This fraction is however considerably less active than the cytosol at least when using the present type of assay. In the cases studied, the microsomal phosphatidic acid phosphatase activity responds in the same direction as the soluble enzyme after various treatments (13). In view of this, only the soluble activity was studied in the present work.

If the phosphatidic acid phosphatase is catalyzing the rate-limiting step in the biosynthesis of triglycerides, an accumulation of phosphatidic acid could be expected to occur under various conditions. Since very little such accumulation occurs, this has previously been taken as evidence against the contention that the phosphatase is of regulatory importance in vivo. It is known, however, that there are active phospholipases in the liver which might dispose excess phosphatidate not converted into diacylglycerol (14). If so, the reactions of phosphatidic acid synthesis and deacylation would constitute a substrate cycle which could remove excess phosphatidate and by returning fatty acids from the unesterified fatty acid pool could provide it with another opportunity to be oxidized (14). Another possibility is that accumulation of phosphatidate within the microsomal membranes may inhibit the acylation of sn-glycerol-3-phosphate, thus preventing further formation of phosphatidic acid (15). In any case, all available evidence obtained from various in vitro experiments is in accord with the contention that phosphatidic acid phsophatase is the rate-limiting enzyme in hepatic triglyceride biosynthesis (9, 13).

The present work clearly demonstrates that the bile acid flux through the liver can regulate phosphatidic acid phosphatase in the same direction as it regulates 7α -hydroxylation of cholesterol. Thus, removal of the bile acids by biliary drainage

or by treatment with cholestyramine slightly increased the activity, whereas treatment with cholic acid and chenodeoxycholic acid markedly decreased the activity. Biliary drainage as well as treatment with cholestyramine in rats stimulates 7α-hydroxylation of cholesterol more than it seems to stimulate phosphatase activity (cf. ref. 8). The inhibitory effect of bile acids on the phosphatase activity was however of the same magnitude as the inhibitory effect of these bile acids on 7α-hydroxylation of cholesterol (cf. ref. 16).

The present findings offer an explanation for the previous in vivo observations of parallel changes in bile acid flux and plasma triglyceride production rate (2-4). Obviously, it cannot be decided presently whether the influence of the bile acids is mediated directly or indirectly. Also, other factors such as substrate availability (17) and the relative amount of diacylglycerol later incorporated into phospholipids may affect net triglyceride synthesis in the liver. In spite of such limitations, however, the present work strongly suggests that changes in bile acid metabolism induce changes in endogenous triglyceride synthesis. Attempts to assay hepatic phosphatidic acid phosphatase activity in humans under various conditions are presently in progress.

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