Estradiol- and testosterone-induced alterations in phosphatidylcholine and triglyceride synthesis in hepatic endoplasmic reticulum

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ABSTRACT Pathways of phosphatidylcholine and triglyceride biosynthesis were studied in hepatic endoplasmic reticulum from castrated and noncastrated male rats pretreated with estradiol or testosterone.

In vitro measurements of hepatic microsomal enzymes which catalyze phosphatidylcholine biosynthesis revealed a significant increase in the specific activity of the enzyme governing phosphatidylcholine biosynthesis by the sequential methylation of phosphatidylethanolamine in the estradiol-treated castrate animals. The specific activity of phosphorylcholine–glyceride transferase was decreased by estradiol treatment in both castrate and noncastrate animals. The specific activity of diglyceride acyltransferase, which catalyzes triglyceride biosynthesis, was decreased by estradiol pretreatment in both castrate and noncastrate animals and was increased by testosterone in the castrate animals.

The changes in specific activity of the enzymes governing phosphatidylcholine biosynthesis may account for the previously noted increased in vivo incorporation of methyl groups of L-methionine into hepatic phosphatidylcholine in female and estradiol-treated animals; the data suggest that in female and estradiol-treated rats a greater proportion of hepatic phosphatidylcholine is synthesized by the stepwise methylation of phosphatidylethanolamine. The decrease in diglyceride acyltransferase specific activity seen after estradiol administration may account for the lipotropic-like effect of estradiol.

SUPPLEMENTARY KEY WORDS *S*-adenosyl-L-methionine:phosphatidylethanolamine methyltransferase · phosphorylcholine–glyceride transferase · diglyceride acyltransferase · choline · L-methionine · CDP-choline · palmitoyl CoA · D-1,2-diglyceride

It is well established that there are differences between male and female rats with regard to the hepatic biosynthesis of certain complex lipids. Investigations by Natori (1), by Bjørnstad and Bremer (2), and by Lyman et al. (3) indicate an increased in vivo incorporation of methyl-^{3}H from L-methionine-methyl-^{3}H into hepatic phosphatidylcholine in female rats. Lyman et al. (4) noted a similar increase in in vivo incorporation of ^{3}H from L-methionine-methyl-^{3}H into hepatic phosphatidylcholine after administration of estradiol to castrated male rats. In the livers of female rats, fatty acid patterns in the phosphatidylcholine fractions with the highest ^{3}H incorporation were similar to those of phosphatidylethanolamine (4). These studies suggest that in the female rat a greater proportion of hepatic phosphatidylcholine is synthesized by the stepwise methylation of phosphatidylethanolamine.

Administration of estradiol prevents to a large extent the hepatic accumulation of triglyceride in the choline- and protein-deficient rat (5–8). However, the effect of estradiol appears to differ from the lipotropic effect of choline, methionine, and Vitamin B_{12} (7, 8).

Previous studies indicating sex differences in the metabolism of complex lipids of the liver have largely described either alterations in the in vivo incorporation of precursors or alteration in the quantity of accumulated hepatic lipids. The enzymes which govern the hepatic biosynthesis of phosphatidylcholine and triglyceride are located in the endoplasmic reticulum (9, 10). Changes in the activity of enzymes governing the biosynthesis of these lipids after the administration of sex hormones have not been described.

The present report describes the effect of administra-

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tion of estradiol and testosterone on the specific activity of the hepatic microsomal enzymes CDP-choline: 1,2-diglyceride cholinephosphotransferase (EC 2.7.8.2) (phosphorylcholine–glyceride transferase), which catalyzes the biosynthesis of phosphatidylcholine (3-*sn*-phosphatidylcholine) from D-1,2-diglyceride (1,2-diacyl-*sn*-glycerol) and CDP-choline (cytidine diphosphate choline); *S*-adenosyl-L-methionine:phosphatidylethanolamine methyltransferase (SAME:PE methyltransferase), which catalyzes the biosynthesis of phosphatidylcholine by the sequential methylation of phosphatidylethanolamine using *S*-adenosyl-L-methionine as the methyl donor; and acyl CoA:1,2-diglyceride *O*-acyltransferase (EC 2.3.1.20) (diglyceride acyltransferase), which catalyzes the biosynthesis of triglyceride (triacylglycerol) from D-1,2-diglyceride and acyl coenzyme A. Alterations in the specific activities of these enzymes which utilize D-1,2-diglyceride as a common precursor have been noted in conditions of altered hepatic lipid metabolism (11) and in association with the proliferation of the endoplasmic reticulum induced by phenobarbital (12).

METHODS

Materials

Choline-1,2-^{14}C bromide (1.03 mCi/mmole) was obtained from Tracerlab, Waltham, Mass. Palmitic acid-1-^{14}C (10 mCi/mmole) and *S*-adenosyl-L-methionine-methyl-^{14}C (42 mCi/mmole) were obtained from New England Nuclear Corp., Boston, Mass. The purity of the palmitic acid-1-^{14}C was checked by thin-layer chromatography. *Clostridium perfringens* type A toxin, *S*-adenosyl-L-methionine, and cytidine-5′-monophosphate were obtained from Sigma Chemical Co., St. Louis, Mo. Coenzyme A (CoA) was obtained from C. F. Boehringer and Sons, Mannheim, Germany. Silica gel G and silica gel H were obtained from E. Merck A.G., Darmstadt, Germany. Reference lipids were obtained from Applied Science Laboratories Inc., State College, Pa.

Estradiol benzoate was obtained from the Schering Corp., Bloomfield, N.J. Testosterone propionate was obtained from the Ciba Pharmaceutical Co., Summit, N.J.

Cytidine diphosphate choline-1,2-^{14}C and palmitoyl-1-^{14}C CoA were chemically synthesized as previously described (11). Diglyceride was prepared from egg yolk phosphatidylcholine as previously described (11). All organic solvents were freshly distilled using glass stills.

Animals

Weanling male rats of the Osborne-Mendel strain were divided into two groups of 24 rats each. All rats of one group were castrated. All rats were raised on ground lab chow and housed in individual cages with controlled temperature and humidity. When weights of the rats reached 100–105 g, both castrate and noncastrate groups were divided into three subgroups of eight rats each. Animals in a subgroup of both castrate and noncastrate groups received subcutaneous injections of 50 μg of estradiol benzoate in 0.2 ml of sesame oil, 1 mg of testosterone propionate in 0.2 ml of sesame oil, or 0.2 ml of sesame oil alone. Injections were given every other day for a total of nine injections (17 days).

Dietary Management

To ensure equal food intake and to diminish the possibility of any enzyme induction or repression resulting from varying caloric intake, the subgroups within each group were fed isocaloric, weighed amounts of ground lab chow. In each group the subgroups ingesting the smallest quantity of food determined the amount given the other two groups. Animals were allowed free access to water.

Analytical Procedures

Microsomal protein was determined on fresh microsomal preparations by the method of Lowry et al. (13). Microsomal neutral lipids and phospholipids were isolated by ethanol extraction using the method previously described for neutral lipid and phospholipid isolation from enzyme assays (11, 12).

For determination of radioactivity of individual phospholipids, aliquots of the total phospholipid extracts were chromatographed on silica gel G thin-layer plates as previously described (11). The areas containing lysophosphatidylcholine, sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine were scraped off and counted in a Packard Tri-Carb scintillation counter using toluene–Cab-O-Sil solvent with 2,5-diphenyloxazole and 2,2-*p*-phenylenebis(5-phenyloxazole) as scintillators. 95% of total phospholipid counts were always found in the phosphatidylcholine fraction. The remaining counts were present largely in the lysophosphatidylcholine fraction. For determination of radioactivity of neutral lipids, aliquots of neutral lipid extracts were chromatographed on silica gel G thin-layer plates as previously described (11). Quenching was monitored by the channels ratio method. Quenching curves were prepared by the addition of phosphatidylcholine and triglyceride, containing known amounts of radioactivity, to the counting solvent with variable, small amounts of silica gel G.

After the isolation of microsomal phosphatidylcholine by thin-layer chromatography on washed silica gel H, phosphatidylcholine phosphorus was determined by the method of Parker and Peterson (14).

TABLE 1 EFFECTS OF ESTRADIOL AND TESTOSTERONE PRETREATMENTS

	Castrate			Noncastrate		
	Control	Estradiol	Testosterone	Control	Estradiol	Testosterone
Liver weight (g)	5.1 ± 0.41	4.2 ± 0.35*	5.5 ± 0.42†	5.6 ± 0.45*	4.3 ± 0.42*	5.5 ± 0.64†
Total microsomal protein (mg)	90.70 ± 5.2	75.39 ± 6.7*	98.34 ± 5.0†	104.7 ± 6.5	78.3 ± 5.3*	94.80 ± 6.8†
Total microsomal phosphatidylcholine P (μmoles)	10.5 ± 0.42	9.0 ± 0.40*	10.1 ± 0.39†	12.3 ± 0.54	8.9 ± 0.38*	11.7 ± 0.45†

Control and sex hormone-treated groups consisted of eight animals each. All values represent means ± SD.
* Significantly different from control values ($P < 0.01$).
† Not significantly different from control values.

Enzyme Preparations

On the morning of the 18th day the rats were killed by decapitation, and the livers were excised, weighed, rapidly cooled, and minced; they were then homogenized in 5 vol of ice-cold 0.25 M sucrose containing 0.001 M EDTA by means of a Potter-Elvehjem homogenizer with a Teflon pestle. Microsomes were isolated from the crude homogenate as previously described (11). Phosphorylcholine–glyceride transferase was assayed as previously described (11), using enzyme-saturating concentrations of both CDP-choline-1,2-^{14}C and D-1,2-diglyceride. Diglyceride acyltransferase activity was assayed as previously described (11), using palmitoyl-1-^{14}C CoA and D-1,2-diglyceride as substrates in enzyme-saturating concentrations. SAME:PE methyltransferase activity was assayed by a modification of the method of Bremer and Greenberg (10), as previously described (12). All animals were killed within a 120-min period to avoid diurnal variations in lipid metabolism (15).

RESULTS

Effect of Sex Hormones on Food Intake and Weight Gain

During the first 7 days of the hormone-injection period, the estradiol subgroups in both the castrate and noncastrate groups ate slightly less food than the testosterone-treated and control subgroups. Thus, the food intake of these animals limited the food intake of the other two subgroups. During the remaining 10 days, however, food intake of both the castrate and noncastrate control and estradiol-treated groups was equal, and the intake of these groups determined the intake of the testosterone-injected groups. Although the animals were fed isocalorically, the average daily weight gain of both the castrate and noncastrate rats receiving estradiol was significantly less than that of their respective control groups. In the castrate group the estradiol-treated animals gained 3.8 ± 0.21 g per day compared with an average daily gain of 4.8 ± 0.2 g per day for the control animals ($P < 0.01$). In the noncastrate group the difference in daily weight gain between estradiol-treated and control animals was slightly greater, with gains of 3.7 ± 0.26 g per day and 5.3 ± 0.23 g per day, respectively ($P < 0.01$). The average daily weight gain of the testosterone-treated castrate animals was greater than the control castrate animals, being 5.5 ± 0.22 g per day ($P < 0.01$), and thus greater than the estradiol-treated animals ($P < 0.001$). The average daily weight gain in the noncastrate testosterone-treated rats was not significantly different from their control subgroup.

Effect of Estradiol and Testosterone Administration on Microsomal Protein and Phosphatidylcholine Levels

The effect of estradiol and testosterone pretreatment on liver weight, hepatic microsomal protein, and microsomal phosphatidylcholine levels are shown in Table 1. Liver weights decreased in the estradiol-pretreated animals in both the castrate and noncastrate groups. The decreased liver weight in the estradiol-treated animals corresponds in direction and magnitude to the decreased weight gain noted in this group. Corresponding to the decrease in liver weight, there was a decrease in total microsomal protein and total microsomal phosphatidylcholine. The ratios of total microsomal protein to total microsomal phosphatidylcholine were not significantly different in the control, estradiol-, or testosterone-treated subgroups in either castrate or noncastrate animals. Adminstration of testosterone had no significant effect on liver weight, total microsomal protein, or total microsomal phosphatidylcholine when compared with control animals. Because animals in each subgroup were isocalorically fed, it is unlikely that difference in food intake accounts for the changes noted. Although the testosterone-treated animals in both castrate and noncastrate groups had their food intake limited initially by the estradiol-treated group and later by both estradiol-treated and control animals, the weight of the liver and levels of microsomal protein and phosphatidylcholine of the testosterone-treated subgroups were not significantly different from the values for the control animals in their respective groups.

TABLE 2 SPECIFIC ACTIVITIES OF HEPATIC MICROSOMAL ENZYMES FROM CASTRATE AND NONCASTRATE MALE RATS AFTER 17-DAY PRETREATMENTS WITH ESTRADIOL AND TESTOSTERONE

Enzyme	Castrate			Noncastrate		
	Control	Estradiol	Testosterone	Control	Estradiol	Testosterone
SAME:PE methyltransferase specific activity*	0.396 ± 0.031	0.559 ± 0.036†	0.371 ± 0.046‡	0.325 ± 0.035	0.386 ± 0.053‡	0.316 ± 0.036‡
Phosphorylcholine-glyceride transferase specific activity*	11.5 ± 0.8	9.8 ± 0.9§	11.6 ± 1.1‡	12.3 ± 0.9	9.4 ± 1.4§	12.2 ± 1.1‡
Diglyceride acyltransferase specific activity*	8.7 ± 0.30	5.1 ± 0.34†	11.3 ± 0.52§	10.3 ± 0.52	6.6 ± 0.38†	11.1 ± 0.6‡

Control and sex hormone-treated groups consisted of eight animals each. All values represent means ± SD.
* Specific activity expressed as nmoles of labeled substrate incorporated per min per mg of protein.
† Significantly different from control values ($P < 0.01$).
‡ Not significantly different from control values.
§ Significantly different from control values ($P < 0.02$).

Enzyme Activities

The effects of administration of estradiol and testosterone on the specific activities of SAME:PE methyltransferase, phosphorylcholine–glyceride transferase, and diglyceride acyltransferase are seen in Table 2. In the castrate animals, estradiol resulted in a significant increase in the specific activity of SAME:PE methyltransferase. Although a moderate increase in the activity of this enzyme was noted in the noncastrate estradiol-treated group when compared with its control, the variation in specific activity in this subgroup made the difference not significant. Previous investigations by Bremer and Greenberg (10) have indicated that the addition of mono- and dimethylated phosphatidylethanolamine increases the rate of incorporation of methyl-^{3}H from L-methionine-methyl-^{3}H into phosphatidylcholine when SAME:PE methyltransferase activity is assayed in vitro. Addition of phosphatidylethanolamine was without effect. In the present investigations, microsomal concentrations of these individual methyl acceptors were not determined, so an increased synthesis of only one of these methyl acceptors cannot be distinguished from an increase in the activity of the entire pathway. The specific activity of phosphorylcholine–glyceride transferase was decreased in both the castrate and the noncastrate estradiol-treated animals. Testosterone administration over the 17-day period caused no change in the specific activity of SAME:PE methyltransferase or of phosphorylcholine–glyceride transferase in either the castrate or the noncastrate groups.

Diglyceride acyltransferase specific activity was decreased in both castrate and noncastrate estradiol-treated animals compared with their respective control groups.

The administration of testosterone to the castrate animals resulted in an increase in the specific activity of diglyceride acyltransferase. This effect was the opposite of that noted after administration of estradiol. Testosterone caused no change in diglyceride acyltransferase in the noncastrate animals.

DISCUSSION

The present studies indicate that the administration of estradiol to both castrate and noncastrate male rats has a significant effect on the specific activity of enzymes governing the biosynthesis of both phosphatidylcholine and triglyceride. After testosterone administration, a significant effect on the specific activity of diglyceride acyltransferase was noted only in the castrate male rat. The increase in the specific activity of SAME:PE methyltransferase noted in the castrate animals after estradiol administration is consistent with the increased in vivo incorporation of methyl groups from L-methionine noted in female rats by Natori (1), Bjørnstad and Bremer (2), and Lyman et al. (3), and as noted by Lyman et al. (4) after estradiol administration to castrate male rats. A comparable change in the specific activity of SAME:PE methyltransferase was not seen in the noncastrate group.

The decreased rate of weight gain noted in both the castrate and noncastrate estradiol-treated animals is in agreement with observations by others (7, 8, 16). Although all subgroups were isocalorically fed, this decrease in rate of weight gain was associated with decreased liver weight and microsomal protein level. It is unlikely, however, that the alterations in enzyme specific activity are causally related to these changes. As noted in Table 2, the specific activity of SAME:PE methyltransferase increased in the castrate animals while the specific activities of phosphorylcholine–glyceride transferase and diglyceride acyltransferase were decreased to different degrees in both groups.

The decrease in specific activity of phosphorylcholine–

glyceride transferase noted in both estradiol-treated groups is an effect opposite to that noted with respect to SAME:PE methyltransferase. Thus, the estradiol-treated animals might be expected to have a relative decrease in overall de novo phosphatidylcholine biosynthesis by the pathway catalyzed by phosphorylcholine–glyceride transferase while that occurring by the stepwise methylation of phosphatidylethanolamine is increased.

It is of interest that in the investigations reported by Lyman et al. (4), the change in fatty acid composition of phosphatidylcholine after estradiol administration (a decrease in linoleic acid and an increase in long-chain polyunsaturated [>20:4] fatty acids) also occurred in phosphatidylethanolamine to a similar extent. This suggests that estradiol administration to castrated male rats results in significant enzyme changes in addition to the increased specific activity of SAME:PE methyltransferase noted in the present investigation. The alterations of fatty acid patterns might be explained by an increase in acyl exchange in phosphatidylethanolamine and phosphatidylcholine due to altered activity of hepatic phospholipase (17) and the enzymes governing acylation of lysophosphatidylcholine (18) and lysophosphatidylethanolamine (19).

The decreased diglyceride acyltransferase specific activity noted in both castrate and noncastrate estradiol-treated animals may account in part for the lipotropic effect noted by Gyorgy, Rose, and Shipley (5), Gyorgy and Rose (6), Plagge, Marasso, and Zimmerman (7), and Bowser, Henderson, and Zimmerman (8). Preliminary studies in this laboratory on the choline-deficient rat (20) have shown a significant increase in the specific activity of hepatic microsomal diglyceride acyltransferase in rats fed a choline- and protein-deficient diet when compared with choline-supplemented controls. Thus, the decrease in the specific activity of diglyceride acyltransferase after estradiol administration noted in the present investigations suggests that estradiol may exert its lipotropic-like effect in part by decreasing the rate of triglyceride biosynthesis in the hepatic endoplasmic reticulum.

Previous investigations in this laboratory (12) have indicated that the specific activity of SAME:PE methyltransferase is increased in association with induction of proliferation of the hepatic endoplasmic reticulum after phenobarbital administration. The specific activity of diglyceride acyltransferase after phenobarbital administration was increased to a degree comparable to the increase in specific activity found in the phenobarbital-induced drug-metabolizing enzyme which oxidatively demethylates aminopyrine. Although in the present investigations the specific activity of SAME:PE methyltransferase is noted to be comparably increased in the castrate estradiol-treated rat, several factors indicate that this increased specific activity is not related to the same mechanism. As noted in Table 1, liver size and total microsomal phosphatidylcholine and total microsomal protein were significantly decreased in the estradiol-treated animals from both the castrate and noncastrate groups. These changes are opposite to those noted after phenobarbital administration. Moreover, after estradiol administration diglyceride acyltransferase specific activity was significantly decreased, whereas after phenobarbital administration the specific activity of this enzyme was increased.

Studies by Mukherjee and Bhose (21) on the regulation of cholesterol biosynthesis in male rats indicate a significant decrease in the specific activity of both hepatic hydroxymethylglutaryl CoA condensing enzyme and hydroxymethylglutaryl CoA reductase after 17β-estradiol administration. The alterations in these enzymes in conjunction with the alterations in SAME:PE methyltransferase and diglyceride acyltransferase may contribute to the altered serum patterns of cholesterol and phospholipid as well as to the altered lipoprotein patterns seen in humans after the administration of estrogenic hormones.

From the present studies it is clear that the enzymes SAME:PE methyltransferase, phosphorylcholine–glyceride transferase, and diglyceride acyltransferase are, in the broad sense, induced and repressed by the administration of the sex hormones. It is not clear, however, that these hormones are the effector substances directly responsible for the changes in the enzyme specific activities noted. The administration of sex hormones may result in many metabolic changes which might affect rates of synthesis of complex lipids.

The in vitro enzyme assays carried out in these investigations are designed only to evaluate the direction and the magnitude of changes in enzyme specific activity. They are not intended to indicate maximum synthetic rates which might be found in vivo. These enzyme studies do, however, suggest that the administration of the sex hormones estradiol and testosterone alters the hepatic biosynthesis of both phosphatidylcholine and triglyceride.

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