Communication

Estrogen Treatment Increases Phospholipid Transfer Activities in Chicken Liver*

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The effect of subcutaneous β-estradiol injection on liver phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol transfer activity of immature chicken has been determined. The estrogen administration significantly enhanced the transfer activity of both phosphatidylcholine (100%), phosphatidylethanolamine (160%), and phosphatidylinositol (150%). In vivo experiments revealed that the hormone-induced changes in liver lipid transfer activity were sensitive to a protein synthesis inhibitor, cycloheximide. A partial characterization of liver protein transfer on Sephacryl S-200 showed that multiple transfer proteins are involved in the β-estradiol effect.

This is the first time that hormonal modulation of phospholipid transfer activities is described, and the results suggest that the hepatic phospholipid transfer activities might be involved in the biosynthesis of plasma lipoproteins in vivo.

Lipid transfer proteins are widely distributed among animals, plants, other eukaryotic, and also prokaryotic cells. Several of these proteins have been purified, characterized, and used as probes in a variety of studies concerning the structure and function of natural and artificial membranes (1-6). Despite the wealth of information on these proteins and their use as tools in vitro, there is a large void in understanding their physiological functions. Experiments performed with lipid transfer proteins purified from bovine liver showed that some of them catalyze the transfer of labeled phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine as well. A portion of these phospholipids is assembled with triacylglycerides and apoproteins early in the secretory pathway, and the remaining seems to be introduced just prior to its secretion as mature lipoprotein (16). It occurred to us that estrogenized chicken could be used to explore the eventual role of lipid transfer proteins in the biosynthesis of hepatic lipoproteins in vivo.

In this paper, we describe a significant induction of lipid transfer activities by estrogens and the inhibitory effect of cycloheximide, suggesting that the estrogen effect could be tightly coupled to the rate of these proteins’ synthesis.

MATERIALS AND METHODS

Animals—White Leghorn pullets (100-125 g) were fed a standard chicken diet and subjected to 14 h of light/day. Experimental animals were given subcutaneous injections of β-estradiol (2 mg/100 g of body weight) and in some cases plus cycloheximide (0.5 mg/100 g of body weight, Sigma) dissolved in 0.2 ml of propylene glycol. Control birds were injected with propylene glycol only. Injections were given at the beginning of the experiments and 24 h later. Forty-eight h after the beginning of the experiment, the birds were killed by exsanguination through severed cervical blood vessels. Blood was collected in beakers containing EDTA (10%, w/v; 1 volume/10 volumes of blood), and the plasma was separated by centrifugation and subjected to lipid extraction and analysis. Livers were rapidly excised and placed in beakers containing ice-cold Tris buffer (25 mM Tris/HCl, 150 mM NaCl 1 mM EDTA, 5 mM β-mercaptoethanol, 3 mM NaN3, pH 7.4). After removal of any nonhematopoietic tissue, they were weighed, cut into small pieces, and washed several times with buffer. A 30% homogenate was prepared in the same buffer, and aliquots were saved for lipid extraction and analysis. The remaining homogenate was centrifuged for 15 min at 15,000 × g, and the supernatant was adjusted to pH 5.1 with 1 N HCl. After 15 min in ice, the samples were centrifuged for 20 min at 15,000 × g to yield a clear supernatant that was adjusted to pH 7.4 and used immediately for determination of phospholipid transfer activities or fractionated by a Sephacryl S-200 column.

Partial Purification of Phospholipid Transfer Proteins from Chicken Liver—The pH 5.1 supernatant was readjusted to 7.4, and solid ammonium sulfate was slowly added to 90% of saturation. The mixture was stirred for 12 h, and the precipitate was sedimented by centrifugation for 20 min at 15,000 × g. The pellet was resuspended in 5 ml of Tris buffer and loaded into a Sephadex G-25 column (1 × 20 cm), eluted at 5 ml/h·cm² with Tris buffer. The void volume was applied onto a Sephacryl S-200 column (2.5 × 100 cm) and eluted at 30 ml/h·cm² with the same buffer. 3.5-ml fractions were collected and used for determination of phospholipid exchange activity.

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† Fellow from Consejo Nacional de Investigaciones Cientificas y Tecnicas (Argentina), now deceased.

§ Career investigator of Consejo Nacional de Investigaciones Cientificas y Tecnicas (Argentina).
Lipids and Lipid Vesicles—The aliquots of plasma and liver homogenates were subjected to Folch’s extraction (17). Triacylglycerides were determined according to Royer et al. (18), and cholesterol was determined by the method of Huang et al. (19), using for both commercially available kits (Wiener Laboratories, S.A.I.C. Rosario, Argentina). Lipid phosphorus was determined by the method of Ames and Dubin (20). The lipids were also analyzed by thin layer chromatography.

\[^{14}C\]PtdCho, \[^{14}C\]PtdEtn, and \[^{14}C\]PtdIns were obtained from soybean seed as described in detail elsewhere (21). The phospholipids exhibited a radiopurity greater than 97% checked by thin layer chromatography. The specific activities ranged between 0.15 and 0.50 μCi/μmol, Du Pont-New England Nuclear) was purified by Silica Gel H thin layer chromatography as described previously (21). Vesicles containing egg yolk PtdCho and [\(^{14}C\)]PtdCho, [\(^{14}C\)]PtdEtn, and [\(^{14}C\)]PtdIns (molar ratio, 1/1) and a trace of [\(^{3}H\)]triolein were prepared using a sonicing water bath as described before (21).

Phospholipid Transfer Activities—Phospholipid transfer activities were determined by measuring the transfer of \[^{14}C\]labeled phospholipids from sonicated vesicles to cross-linked erythrocyte ghosts as described previously (21). Protein concentration was determined according to Bradford (22) with bovine serum albumin as a standard, and transfer activities were expressed as percent of transfer per h per mg of protein.

RESULTS

Inductions of avian hepatic lipogenesis and plasma lipids by estrogen have been described previously (15). Administration of diethylstilbestrol (40 mg/kg of body weight) doubled the plasma phospholipid in immature male turkeys after 48 h.

TABLE I
Liver phospholipid transfer activity following estrogen treatment of immature chicken

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<th>%/h/mg protein</th>
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<tr>
<td>Control</td>
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</tr>
<tr>
<td></td>
<td>PtdIns</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>PtdEtn</td>
<td>1.0 ± 0.10</td>
</tr>
<tr>
<td>Treated</td>
<td>PtdCho</td>
<td>10.8 ± 1.2*</td>
</tr>
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<td></td>
<td>PtdEtn</td>
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\*p < 0.01.
\#p < 0.02.

FIG. 1. Time course of PtdCho transfer activities. Control chicken (△), injected with \[^{14}C\]estradiol (2 mg/100 g of body weight) (●), and injected with \[^{14}C\]estradiol (2 mg/100 mg of body weight) and cycloheximide (0.5 mg/100 g of body weight) (□) were killed at different times. The pH 5.1 supernatant from liver was obtained, and its PtdCho transfer activity was measured as described under “Materials and Methods.” Values are mean of three to seven experiments.

FIG. 2. Elution profiles of Sephadex G-25-treated pH 5.1 supernatant from chicken liver on Sephacryl S-200. The pH 5.1 supernatants from control (□) and treated (○) chicken were obtained as described under “Materials and Methods” and applied to a Sephacryl S-200 column. Fractions of 5 ml were collected and assayed for phospholipid transfer activities. A, PtdCho transfer activity; B, PtdIns transfer activity; C, PtdEtn transfer activity.

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The values are the mean of nine chickens/group ± S.D.; the values of the two groups were significantly different.

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of a single injection. In our experiments with chicken, the same total amount of \[^{13}C\]estradiol, administered in two separate injections, resulted in an averaged 5.6-, 16-, and 3.5-fold increase in plasma phospholipids, triacylglycerides, and cholesterol, respectively. Compared with plasma, hepatic neutral lipids were less increased in estrogen-treated birds (40%). On the other hand, reproducible averaged 100% increase in liver phospholipid content was found (data not shown).

In clear contrast with mammals, avian liver has been little studied with respect to phospholipid transfer activities. PtdCho and sphingomyelin transfer activities have only recently been described in postmicrosomal supernatant of
chicken liver using a liposome-mitochondria assay (19). We have confirmed the existence of PtdCho transfer activity in the PtdIns and PtdEtn transfer activity. Moreover, we have previously determined PtdCho transfer activities in pH 5.1 supernatant of rat liver (21), and a value of 3.7 (percent transfer per h per mg of protein) was obtained. An averaged value of 4.9 (same units) was found in the present work for chicken liver supernatant. As shown in Table I, the highest activity was for PtdCho followed by PtdIns; the lowest transfer activity was for PtdEtn. Similar trends were previously observed in rat (23) and beef liver (24). After 48 h of the estrogen treatment, the level of PtdCho, PtdEtn, and PtdIns increased 2.2., 2.-, and 2.5-fold, respectively. In spite of the variability in the degree of stimulation among the separate experiments, the activity increase due to estrogen is clearly significant (Table I).

Phospholipid transfer activities were measured as a function of time following estrogen injection (Fig. 1). The PtdCho activity was 30% increased as early as 6 h after injection. The maximum effect of estrogen was achieved 48 h after treatment (110% increase). In contrast, control chickens injected without estrogen did not significantly change the PtdCho activity. A similar time dependence curve was obtained with PtdIns and PtdEtn activities (data not shown). The increase of phospholipid transfer activity could be due to an activation effect or protein synthesis stimulation. Since cycloheximide is a potent inhibitor of protein synthesis, we have tested the effect of cycloheximide on the change of transfer activity. Chicken was treated with cycloheximide and estrogen (Fig. 2). The PtdCho transfer protein was induced in the same extension. Possibility requires further investigation.

In the present study we have clearly demonstrated that an increase in chicken liver of phospholipid transfer activity occurred after estrogen injection. Furthermore, an inhibitor of protein synthesis such as cycloheximide inhibited the effect. This observation strongly suggests that estrogen triggered the induction of lipid transfer protein synthesis. The possibility that estrogen induced a nonspecific lipid transfer protein has been ruled out by analysis of pH 5.1 supernatant on gel filtration (Fig. 2). It seems likely that all the lipid transfer protein was induced in the same extension.

The simultaneous induction of hyperlipidemia, appearance of apolipoprotein E and apolipoprotein B in blood, and the increase of lipid transfer activity after estrogen treatment strongly suggests a close relationship between lipoprotein biosynthesis and phospholipid transfer activity. However, this possibility requires further investigation.

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REFERENCES