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Estrogen Binding in Rat Liver During Pregnancy

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Estrogen binding in the liver of pregnant rats has been studied. When the results are expressed in pmol/mg of protein there is a marked decrease in relation to control rats on 12th or 21st days of gestation. In spite of the liver weight increase on day 21, however, the binding capacity for estrogens in the whole liver is still lower than in controls. It is suggested that the changes in rat liver estrogen receptors in late gestation cannot explain the action of estrogens on triglyceride rich lipoprotein synthesis and the consequent hypertriglyceridemia of late gestation.

Key words: Estrogen, Pregnancy, Liver, Rats.

Two distinct metabolic phases can be distinguished in rat pregnancy (16). In early gestation the foetus has a slow growth pattern and the mother uses a large amount of glucose which is then transformed into fatty acids. In late gestation the foetus grows at a faster rate, consuming an increasing amount of glucose to fulfil its energy needs. When this occurs, the mother shuts off the conversion of glucose into fatty acids and mobilizes the fat previously stored in order to fulfil her own metabolic needs (15). Therefore, at the end of pregnancy there is a disturbance in lipidic metabolism with a remarkable increase in circulating triglycerides, demonstrated in rats (15, 24) and in humans (15, 26).

The hormonal basis for this hypertriglyceridemia in late gestation is not presently known, but it is noteworthy that estrogen treatment enhances the hepatic production of very low density lipoproteins (4, 13, 17). On the other hand, the action of estrogen on hepatic cells is preceded by the binding to specific cytoplasmatic proteins (6, 22) and the induction of specific proteins by estrogens, such as renin substrate in rats, is related to the translocation of the receptor to the nucleus (14).

In this study we have tried to demonstrate the presence of specific receptors for estrogens in hepatic cells of rats during pregnancy and to look for changes in relation to the two metabolic phases of rat gestation.

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Materials and Methods

Wistar female rats that weighed 200-250 g at mating were used. The rats were allowed *ad libitum* access to food and water until the time of their sacrifice. Day one of gestation refers to the day after the night of breeding. Rats of an identical weight at the time of breeding were used as nonpregnant controls or for estrogen treatment. Estradiol benzoate dissolved in an oleic vehicle was administered by intramuscular injection, one or two mg per animal.

Rats were anesthetized with ether and the liver perfused with normal saline through the vena cava, with a variable of 9-21 ml/min, using a LKB Multiperpex perfusion pump. The liver was then quickly excised and retained in ice.

Cytosol preparation. — All subsequent steps were performed at 0-4°C. The liver was washed twice in saline and homogenized in 6 volumes (wt/vol) 0.01 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose, using a politron (Kinematica). The homogenates were centrifuged at 800 *g* for 20 min in a Beckman L5-50 ultracentrifuge, using a 50 Ti rotor. The supernatant of this centrifugation were then centrifugated at 105,000 *g* 90 min and the clear supernatants retained as the cytosol fractions.

Ammonium sulphate precipitation of liver cytosol. — The estradiol binding sites of liver cytosol were partially purified by ammonium sulphate precipitation (2). A saturated solution of ammonium sulphate was added to liver cytosol in a 2:1 proportion (v:v), after having been stirred for 15 min. The mixture was centrifuged at 30,000 *g* for 20 min. The supernatants were discharged and the precipitates redissolved in Tris-HCl buffer.

Hepatic nuclear preparation. — To obtain a nuclear fraction from rat liver the precipitates from the first centrifugation (800 *g*, see above) were redissolved in 5 ml of Tris-HCl buffer, containing 0.25 M sucrose and 3 nM $CaCl_2$, and then centrifuged at 47,000 *g* for 30 min. The precipitates were redissolved in the same buffer and retained as the nuclear fraction.

Uterine cytosol preparation. — Measurement of the binding capacity of uterine cytosol was performed only on control animals. The cytosol fraction, once the uterus was freed from tissues, was prepared by the same process followed to obtain the hepatic cytosolic fraction.

Exchange assays of cell fractions. — Aliquots (0.2 ml) of the total or partially purified liver cytosol, uterine cytosol or liver nuclear fractions, were added to two sets of tubes containing ^{3}H-estradiol with and without an excess of estradiol. After having been vortexed, both sets of tubes were incubated at 0°C for 16 hours. At the end of the incubation period a volume equal to its content of 0.02% dextran-0.25% charcoal in 0.01 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose was added to each tube. The tubes were incubated in ice for 30 min, being gently shaken several times during this period, and then centrifuged at 3,000 rpm for 10 min. After centrifugation, 0.2 ml aliquots of the clear supernate were suspended in 2:1 toluene-triton X-100 (v:v) for counting. The exchange assay results are expressed as specific estradiol binding.

Protein analysis. — Protein was determined by the LOWRY method (20), using BSA as standard.

Lipid analysis. — Plasma from fed rats was ultracentrifuged in order to separate quilomicrons and VLDL (10), and triglycerides were measured in whole plasma and in both lipoprotein fractions by the FLETCHER methods (8), after FOLCH lipid extraction (9).

Results

Estradiol binding in rat liver. — Saturation analysis of total and partially purified cytosolic fractions from livers of control rats was carried out incubating these fractions with different concentrations of ^{3}H-estradiol (1-50 nM) but with the excess of estradiol remaining a constant. Specific binding was determined by the difference between nonspecific and total binding (fig. 1, A and B). The specific binding shows saturation at a ^{3}H-estradiol concentration of 5 nM. Similar experiments carried out with hepatic nuclear fractions and uterine cytosolic fractions showed saturation at a concentration of 30 nM with ^{3}H-estradiol in both cases.

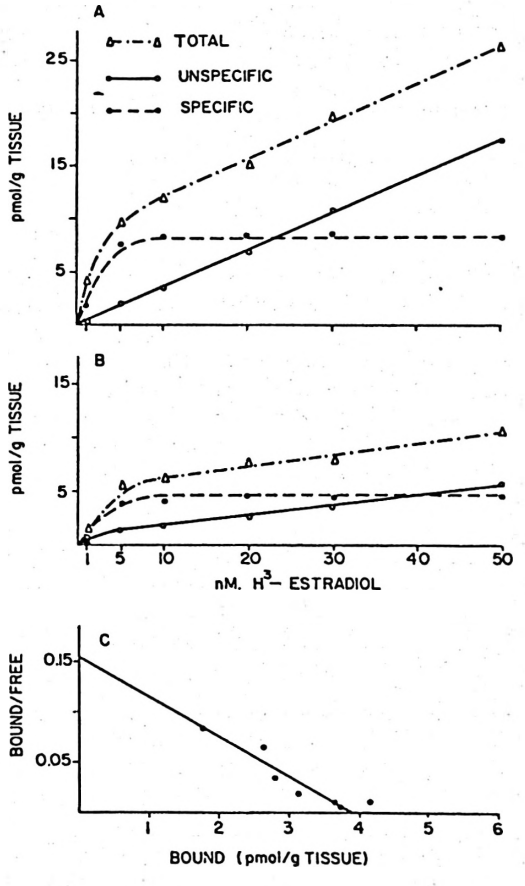

Fig. 1. *Saturation analysis in total (A) and partially purified cytosolic fraction (B) of control rat liver. Scatchard's diagram (C) of the specific binding of estradiol to partially purified cytosolic fraction in control rat liver.*

Table I. *Cytoplasmic estradiol binding in uterus and liver of rats (mean ± SE).*

Organ	N	pmol/g tissue	pmol/mg protein
Uterus	8	8.19±1.23	0.1228±0.001
Liver (total)	51	5.93±0.38*	0.0591±0.004*
Liver (precipitate)	50	3.25±0.24**	0.0986±0.007**

* Liver receptor compared to uterus receptor, $p < 0.001$.

** Receptor in total cytoplasm compared to fraction precipitated with ammonium sulphate at 30%, $p < 0.001$.

Fig. 1-C represents a Scatchard plot of the partially purified liver cytosolic fraction specific binding of control rats. It was obtained incubating this fraction with different concentrations of estradiol, from 25 to 500 nM, with the ^{3}H-estradiol concentration remaining a constant. The plot has a correlation of —0.92 which suggests that there is only an estradiol binding site.

The hepatic cytosol estrogenic receptor was partially purified with 30% ammonium sulphate (2). Following this treatment a double purification was performed which resulted in a 60% recovery (table I). The amount of specific receptors for estrogen in uterus is significantly greater than that found in the liver (table I), and possibly even greater than the values obtained here, as the cytosolic fraction was not washed with charcoal-dextrane to eliminate tissue estrogens. When this washing was done in liver cytosol there was no difference found in the concentration of binding.

The accepted mechanism for esteroid hormones includes the translocation of the hormone-receptor to the nucleus. To check decrease in cytoplasmic binding 1

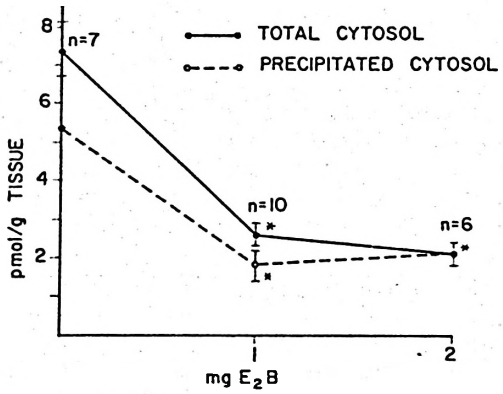

Fig. 2. *Changes in cytoplasmic estradiol binding after estradiol benzoate (E_2B) injection.*
n = number of animals per experiment. Values are means ± SE. $p < 0.001$ (comparison between control rats and treated rats).

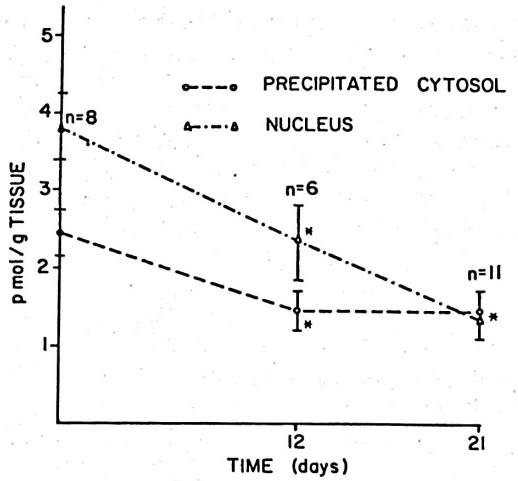

Fig. 3. *Comparison between estradiol binding in nucleus and partially purified cytosol in nonpregnant and pregnant rats.*
n = number of animals per experiment. Values are means ± SE. $p < 0.001$.

or 2 mg of estradiol benzoate was injected in the rats. Two hours after this treatment the binding in totally and partially purified cytosol was 60% lower than in control animals, without any difference in the two doses of hormones (fig. 2).

Changes in cytoplasmic and nuclear estrogen binding in gestation. — The amount of cytoplasmic binding in the partially purified cytosolic fraction is expressed in pmol/g tissue, verifying a decrease in cytoplasmic receptors during pregnancy, with no differences between days 12 and 21 (table II). When the binding is expressed in pmol/mg of protein it results in the same decrease because protein concentration does not change in pregnancy. Due to the significant increase in liver weigth in late gestation, the amount of binding in whole liver shows a light increase at day 21 as compared to day 12, however, those values are lower in both pregnant groups compared to virgin rats.

Table II. *Changes in cytoplasmic estradiol binding, liver weight and total proteins in virgin and pregnant rats (means = SE).*

				Cytoplasmic binding		
Group	N	Liver weight (g)	mg protein/ g tissue	pmol/g tissue	pmol/mg protein	pmol/whole liver
Virgin rats	12	7.77±0.53	34.41±2.84	3.16±0.33	0.0950±0.008	23.91±2.22
Day 12 pregnancy	6	8.42±0.61	35.48±3.36	1.62±0.28**	0.0407±0.004***	13.02±2.85**
Day 21 pregnancy	11	10.92±0.46***	31.58±2.00	1.62±0.23***	0.0447±0.003***	18.24±2.46*

Comparison between pregnant and nonpregnant rats. *** $p < 0.001$ ** $p < 0.01$ * $p < 0.1$.

Fig. 3 shows the changes in hepatic nuclear binding in virgin and pregnant rats compared with the partially purified cytosol preparation. The concentration of nuclear receptors is lower in pregnant rats, and it seems to decrease in a linear manner until day 21, while the cytosolic binding remains the same in both pregnant groups.

Changes in triglycerides in mid and late gestation. — Triglycerides were measured in the plasma of virgin and 21 day pregnant rats. Whole plasma triglycerides increased by four at day 21 (105±0.2 mg/dl vs. 384 ± 0.72). Chylomicron increased from 34 mg/dl to 83 mg/dl and VLDL from 40 mg/dl to 250 mg/dl.

Discussion

The main cause of the hypertriglyceridemia of late gestation in rats is the higher flow in circulating triglycerides (12). This is probably due to an increase in the intake of dietary fat (over 15% at day 21) and an increase in endogenous triglyceride synthesis by the presence of a higher contribution of fatty acids from adipose tissue. These changes are probably due to hormonal modifications. In fact, it has been shown that estrogens increase the synthesis of Apo B in the liver of birds (5) and concentration of fatty acids binding protein in rat liver (27, 28), increasing its uptake by the liver and the synthesis of very low density lipoproteins.

The action of estrogens in specific target cells is dependent upon their binding to a cytoplasmic receptor. Several experimental studies (1, 6) have demonstrated the presence in the cytoplasm of hepatic cells of proteins that specifically bind estradiol. By means of saturation analysis we have verified the existence of a specific binding for the hormone in rat liver cytosol, which reaches saturation from a determined concentration of ^{3}H-estradiol. A partial purification of the cytosolic fraction was performed by ammonium sulphate precipitation, obtaining a 60% recovery, which agrees with the data of ATEN *et al.* (2). This purification is important in order to obtain a reduction in concentration of enzymes that catabolize estradiol and reduction in nonspecific binding (1).

One property of cytoplasmic receptors is that after binding to the specific hormone the complex is translocated to the nucleus; this has been shown for the hepatic estrogens receptor (2). The administration of estradiol benzoate to virgin rats is followed by the decrease in the cytoplasmic binding (fig. 2), but without any difference with two different doses. The existence of a static distribution between cytoplasmic receptors is possible by recycling or new synthesis (25). Several studies have verified changes produced in the hepatic function in rats after the administration of estradiol (3, 18, 23).

The changes in hepatic estrogen receptors in mid-gestation (day 12) and late gestation (day 21) in rats show that the concentration of cytoplasmic binding decreases in both groups when it is expressed in pmol/g of tissue or pmol/mg of protein (table II). In spite of the increase in liver weight at the end of pregnancy, the total number of estrogen receptors in the liver is lower in both gestational groups. This seems to indicate that the concentration of estradiol receptors does not increase during pregnancy on a parallel with total liver proteins. The lower amount of estrogen receptors in the liver during pregnancy can be explained by the translocation to the nucleus. However, nuclear receptors decreased on a parallel to cytoplasmic receptors in both gestational periods.

It has been suggested that the main action of estrogens taking place in the liver is the stimulation of the synthesis and secretion of triglyceride rich lipoproteins (7, 11, 19). However, the increase in

triglyceride concentration and flux in late gestation (12) does not correlate with an increase in the number of estrogen receptors, in spite of an increase in total liver proteins. It is suggested that other hormonal influences may play a role in the hypertriglyceridemia of late gestation.

Resumen

Se estudia en ratas preñadas la unión de los estrógenos al hígado. Cuando los resultados se expresan en pmol/mg de proteína hay un marcado descenso en las ratas de 12 y 21 días de gestación. Sin embargo, a pesar del aumento de peso del hígado al día 21, la capacidad de unión para los estrógenos en el hígado completo aún es menor que en los controles. Se sugiere que los cambios en los receptores para estrógenos en el hígado de rata al final de la gestación explican la acción de los estrógenos sobre la síntesis de lipoproteínas ricas en triglicéridos y la consiguiente hipertrigliceridemia del final de la gestación.

Palabras clave: Estrógenos, Preñez, Hígado, Rata.

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