Different regulation of the concentration of estrogen receptors in the rat liver and uterus following ovariectomy

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Data concerning the short- and longterm effects of ovariectomy on the levels of estrogen binding sites in the rat uterus and liver are presented. The information increases the understanding of the regulation of estrogen receptor synthesis. The circulating estrogen level is suggested to affect receptor synthesis in the uterus and liver differently. Shortly after gonadectomy (2–20 h), an elevation in the concentration of cytoplasmic binding sites in the uterus of 35% was observed, whereas no effect was seen in the liver cell. A longer period of time after ovariectomy (2–3 months) caused a reduction in the number of uterine receptor sites by 74%, whereas in the liver an increase of 84% was detected.

Estrogenreceptor *Ovariectomy* *Rat liver* *Rat uterus* *Receptor synthesis*

1. INTRODUCTION

According to the central dogma concerning steroid hormone action [1,2], the hormone enters the cell by passive diffusion and binds with high affinity to specific receptor proteins in the cytosol. The steroid–receptor complex undergoes a temperature-dependent activation and passes through the nuclear membrane into the nucleus. There it binds to specific chromosomal proteins and/or DNA-sequences causing the stimulation of transcription of specific gene sequences [3–5]. The cytoplasmic receptor pool is then replenished by recirculation of nuclear receptors and by de novo synthesis [6–9]. The hormone is metabolized and excreted.

Estrogen receptors have been detected in both the rat uterus and liver. The physical characteristics of these proteins in the liver are similar to those of the receptor in the uterus and other target organs [10,11]. However, there appear to be differences in the interaction of estrogens with receptors in liver and uterine tissue. This is indicated by the very small changes in concentration of nuclear hormone–receptor complexes in the liver during the estrus cycle [12], whereas in the uterus there is a 3–4-fold increase between the estrus and proestrus stages [13,14]. Furthermore, the regulation of receptor synthesis in these organs seems to be different. In the liver the synthesis of estrogen binding sites is controlled primarily by the hypothalamic–hypophyseal axis after neonatal programming by androgens and estrogens [15,16], whereas the uterine receptor level seems to be closely controlled by the concentration of circulating estrogens and progesterone [17].

This study was carried out to examine the short- and long-term effects of overiectomy on the levels of estrogen binding sites in the uterus and liver. The information derived increases the understanding of the regulation of estrogen receptor synthesis in the two different types of target cells. Results obtained suggest that the circulating estrogen levels affect the receptor synthesis in the liver and uterus differently. Shortly after gonadectomy (2–20 h), an elevation in the concentration of cytoplasmic binding sites in the uterus of 35% was observed, whereas no effect was seen in the liver cell. A longer period of time after ovariectomy (2–3 months) caused a reduction in the number of uterine receptor sites by 74%, whereas in the liver an increase of 84% was detected.

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Table 1

Effect of ovariectomy on animal and liver weight and on liver-protein, DNA and cytosol estrogen receptor content

Time after ovariectomy (h)	Animal weight (g)	Liver wet weight (g)	Cytosolic protein (mg/g liver)	DNA content (mg/g liver)	Receptor content (fmol/mg liver cytosol protein)
0	192 ± 4	8.7 ± 0.3	48.3 ± 1.8	2.92 ± 0.05	18.6 ± 3.3
2	186 ± 1	7.7 ± 0.2	51.6 ± 1.8	2.89 ± 0.08	19.1 ± 2.6
5	193 ± 1	7.8 ± 0.1	42.4 ± 1.4	2.94 ± 0.06	21.1 ± 1.7
20	185 ± 4	6.9 ± 0.2	40.4 ± 1.8	2.91 ± 0.09	24.9 ± 2.0
48	195 ± 5	8.2 ± 0.4	41.4 ± 1.4	2.90 ± 0.03	24.5 ± 3.8
120 (5 d)	205 ± 2	8.8 ± 0.3	37.3 ± 1.3	2.70 ± 0.08	42.2 ± 4.3
336 (14 d)	242 ± 4	10.1 ± 0.3	34.2 ± 1.0	2.56 ± 0.12	39.4 ± 3.2
1440 (60 d)	358 ± 7	11.8 ± 0.5	32.0 ± 1.4	2.91 ± 0.09	45.7 ± 5.7
2160 (90 d)	396 ± 11	11.8 ± 0.3	32.5 ± 1.1	2.90 ± 0.06	42.9 ± 4.0

All values are mean ± SEM

2. EXPERIMENTAL

2.1. *Animals*

Female rats of the Sprague-Dawley strain were used. The animals weighed 190–200 g at the beginning of the experiment. They were kept in a controlled environment at 20°C on regular illumination schedule; lights on at 8 a.m. and off at 5 p.m., and were fed a standard laboratory diet which was provided ad libitum.

Nine groups of rats, consisting of 6 animals each, were used. Operations were carried out under light ether anesthesia. The animals were ovariectomized according to the dorsal approach [18], and left for the time indicated in table 1.

2.2. *Determination of cytoplasmic receptors*

Detailed descriptions of the procedures used are given in [11,16]. The liver was perfused in situ with saline–albumin solution through the portal vein, then rapidly excised, trimmed and weighed. A small portion of the organ (0.5 g) was removed and frozen for DNA-determination and the remaining part homogenized in TE-buffer (0.01 M Tris–HCl–0.0015 M EDTA, pH 7.4 at 0°C) in a Potter-Elvehjelm homogenizer with a Teflon pestle. The homogenate was centrifuged at 82000 × *g* for 30 min, the lipid layer removed and the supernatant recentrifuged at 200000 × *g* for 60 min. Ammonium sulphate was added to the high-speed supernatant to 35% saturation. The precipitate was collected, dissolved in TE-buffer and dialyzed against buffer for 2 h. The dialysate was centrifuged at 150000 × *g* for 30 min and the supernatant used in binding experiments.

The uterus was removed, stripped of adhering fat and connective tissue and weighed. It was then homogenized in TE-buffer (4.0 ml/100 mg tissue) in a glass–glass homogenizer, and the homogenate centrifuged at 800 × *g* for 20 min. The supernatant was recentrifuged at 200000 × *g* for 60 min and the high-speed supernatant used for determination of cytoplasmic estrogen receptors. The nuclear pellet obtained after the first centrifugation was used for DNA-determination. Saturation analysis of specific estrogen cytoplasmic receptor sites was performed with ^{3}H-Moxestrol as radioactive ligand and diethylstilbestrol (DES) as non-labeled competitor according to the dextran-coated method [19]. Binding data was analyzed as in [20], and regression and statistical analyses performed on a Hewlett–Packard computer.

2.3. *Analytical methods*

Protein determination on cytosol fractions was done as in [21] and DNA of hepatic and uterine nuclear fractions determined as in [22] with calf thymus DNA standards. Radioactivity was measured by scintillation counting using Insta-gel (Packard Instrument Co.) as scintillator.

3. RESULTS

Animals were sacrificed at different times after

Table 2

Effect of ovariectomy on uterine weight and protein, DNA and cytosol estrogen receptor

Time after ovariectomy (h)	Uterine wet weight (mg)	Cytosolic protein (mg/g uterus)	DNA content (mg/g uterus)	Receptor content (fmol/mg uterine cytosol protein)
0	267 ± 28	38.7 ± 1.3	2.44 ± 0.20	173 ± 23
2	252 ± 12	40.9 ± 2.4	1.96 ± 0.35	199 ± 14
5	200 ± 19	37.5 ± 2.5	2.22 ± 0.24	236 ± 11
20	177 ± 10	37.1 ± 1.7	2.37 ± 0.03	218 ± 7
48	164 ± 8	30.6 ± 3.3	2.57 ± 0.03	258 ± 9
120 (5 d)	126 ± 17	32.4 ± 2.0	2.53 ± 0.13	249 ± 12
336 (14 d)	72 ± 8	35.5 ± 5.5	4.26 ± 0.22	143 ± 30
1440 (60 d)	74 ± 6	24.2 ± 0.2	4.86 ± 0.14	184 ± 28
2160 (90 d)	82 ± 9	19.9 ± 1.5	4.19 ± 0.24	217 ± 3

All values are mean ± SEM

ovariectomy. Liver wet weight was unaffected by this endocrine manipulation whereas the uterus decreased in weight from 267 mg on day 0 to 164 mg on day 2, and to 82 mg on day 90 (table 1,2). The protein content, expressed as mg protein/g tissue, decreased in both organs as a function of time after gonadectomy. In the liver it decreased by 33% and in the uterus by 49% between day 0 and 90. The DNA content in the liver, expressed as mg/g tissue, did not change during the 90 days after ovariectomy. However, in the uterus a pronounced hypotrophy was seen, the number of cells/organ being decreased by about 65% between day 0 and 90.

The number of receptor sites/liver and uterine cell was estimated from the binding data using 6 pg DNA/cell. On day 0 the concentration of binding sites in the uterine and liver cells were 10640 and 1120, respectively. These values correspond to 173 and 18.6 fmol/mg cytosol protein.

The number of cytoplasmic receptor sites in the uterine cell increased rapidly after ovariectomy (fig. 1) reaching 14140 sites/cell after 2 h and a peak-value of 14350 sites/cell after 5 h. The number of sites then started to decline slowly reaching a pre-operative value of 11490 sites/cell after 5 days. During the time between day 5 and 14 the concentration of receptor sites in the uterine cell was reduced to 4750 sites/cell; i.e., 33% of the peak-level 5 h after ovariectomy. Prolongation of the time after ovariectomy for an additional 76

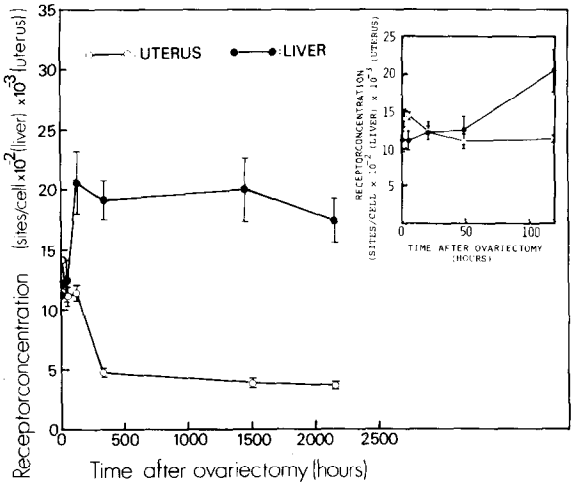

Fig. 1. Concentrations of estrogen receptors in livers and uteri at different times after ovariectomy. Values shown are the mean ± SEM. The inserted figure shows an enlargement of the time period 0–120 h after gonadectomy.

days caused only minor further reductions in the number of binding sites to 3720 sites/cell.

In the liver, very small fluctuations in the concentration of cytoplasmic estrogen binding sites were noted during the first 48 h after ovariectomy. A minor increase from 1120–1250 sites/cell was detected during this period. Between day 2 and 5

the number of binding sites had increased to 2060 sites/cell; i.e., 184% of the initial level on day 0. This increased level was maintained for 85 days after day 5.

4. DISCUSSION

Here the concentration of cytoplasmic estrogen-binding sites in uterine and liver cells were measured at different times after ovariectomy. Plotting the receptor concentration *vs* time after gonadectomy displayed two quite different phases for both. The first was the period immediately after the operation, 0–120 h for the uterus and 0–48 h for the liver; and the second from 120 h–90 days for the uterus and from 48 h–90 days for the liver.

In uterine cells the level of cytoplasmic receptor sites is decreased as a result of nuclear translocation when the circulating level of estrogens is increased. This has been shown both in adult females during the estrus cycle and in immature animals subjected to stimulation with exogenous steroids [13,23–25]. When the endogenous production of estradiol is eliminated in the adult rat by ovariectomy, and the circulating hormone level thus drastically reduced, the level of cytoplasmic binding sites is expected to increase as a result of replenishment of receptors [6,7]. How large this increase will be depends on what stage of the cycle the animal is in [13]. Here an increase of 3700 sites/cell or 35% was detected. This value is very close to those reported in [13] as being the number of nuclear receptor–hormone complexes in the uterine cell during metestrus and diestrus (3500 sites/cell) and proestrus (5000 sites/cell) and which would be expected to be the number of receptor molecules replenished in the cytosol when the animals was gonadectomized.

However, the cyclic changes in the concentration of plasma estradiol during the estrus cycle, which cause nuclear translocation of hormone–receptor complexes in the uterus, are not sufficient to change the receptor distribution between cytosol and nucleus in the liver cell [12]. To achieve nuclear translocation of 50% of the cytoplasmic estradiol–receptor complexes in the liver, an injection of 50–100 μg hormone is required, whereas an injection of only 0.1 μg estradiol causes the corresponding translocation in the uterus. Thus these results, indicating no measureable increase in the number of cytoplasmic receptor sites in the liver during the first 5 h after ovariectomy, accord well with the data in [10,12,26,27].

During the second phase after ovariectomy the uterine weight was reduced by ~70% and the number of cells by 65% compared to the initial values on day 0. The total content of estrogen binding sites in the uterus was reduced by 80% and the concentration in each cell by 74%. These data are in good agreement with the primary purpose of estrogens which is the stimulation of uterine cells to increase in size and numbers [28]. A single injection of 0.1 μg estradiol to immature rats causes a 3-fold increase in the dry weight of the uterus [29]. Thus it can be concluded that the circulating estrogens are the primary regulators of the estrogen receptor content in the rat uterus.

However, long-term gonadectomy did not influence the liver weight. The total content of binding sites in the liver was increased by 210% 3-months after ovariectomy and the cellular concentration by 184%. Thus the circulating estradiol does not seem to affect the synthesis of estrogen binding sites in the liver directly. This observation is in good agreement with reports that the estrogen receptor in the rat liver is under multi-hormonal control and that glucocorticoids in combination with growth hormone and possibly other pituitary factors seem to be responsible for the maintenance of the normal level of receptors [15,16,30]. The 2-fold increased receptor concentration seen 3-months after ovariectomy could be explained by disturbances in the hormonal homeostasis causing a higher output of pituitary hormones than during normal conditions.

This study has shown that the synthesis of the receptor protein for the same hormone in two target organs is primarily controlled by different mechanisms.

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