EFFECT OF ESTRADIOL AND OTHER ENDOCRINE FACTORS ON LEVEL AND DYNAMICS OF ESTROGEN RECEPTORS IN RAT LIVER CELLS

E. V. Gontar', G. D. Mataradze, and V. B. Rozen

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In previous investigations [1] the writers proved that complexes of estradiol (E_2) with cytosol estrogen receptors (ER) of rat liver are translocated *in vitro* into the cell nucleus — the principal site of action of the hormone.

The aim of the present investigation was to discover whether translocation of rat liver cytosol ER takes place into the cell nucleus after injection of various doses of the hormone *in vivo* and, if it does, to study the principles governing the dynamics of ER in the hepatocytes at various time intervals after injection of the hormone. A comparative analysis also was made of changes in the ER level in the hepatocytes on changes on the endocrine status of the animal.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature and immature female albino rats of a mixed population, which were used irrespective of the stage of the cycle. Ovariectomized and hypophysectomized animals were used in the experiments 2-3 weeks after the operation. Various doses of E_2 (from Serva, West Germany) were injected subcutaneously in 0.2 ml of propylene glycol. Somatotrophic hormone (human STH, from Kaunas Endocrine Preparations Factory, activity 1 U/mg) was injected subcutaneously twice a day for 5 days in a dose of 100 μg in 0.2 ml physiological saline. Control animals were given an injection of 0.2 ml of the solvent. Groups of three animals were used. Cytosol and purified nuclei were prepared from liver as described previously [1, 2]. Estrogen receptors in the cytosol (CR) were determined by incubating samples with 7.5 nM [2,4,6,7-^{3}H]-E_2 (specific radioactivity 90-100 Ci/mmole) in the absence (total binding) or in the presence (nonspecific binding) of a 200-fold excess of unlabeled E_2 in the course of 3 h at 0-4°C. Nuclear estrogen receptors (NR) were determined in a 0.4 M NaCl extract of nuclei. When the quantity of NR was calculated a correction was made for completeness of extraction. To determine the NR content the ligand exchange method was used. Aliquots of nuclear extract were incubated with [^{3}H]-E_2 (15 nM) in the presence and absence of a 200-fold excess of unlabeled hormone for 18 h at 0-4°C. Ligand exchange was carried out at 30°C for 60 min. The quantity of specifically bound hormone in the cytosol and nuclear extract was determined by adsorption on dextran-coated charcoal [2]. The content of receptors in the cell was calculated as described in [4]. The statistical significance of the differences between parameters was determined by Student's t test.

EXPERIMENTAL RESULTS

As Table 1 shows, the ER level in the hepatocytes depends on the endocrine status of the animal. The first point to note was that the ER content in hepatocytes of mature female rats was significantly lower than in uterine cells. This fact, together with the rapid hormone metabolism in the liver cells, may evidently raise the threshold of sensitivity to E_2 in certain cases.

A distinguishing feature of reception of E_2 in the liver was a marked increase in the ER content in both cytosol and nuclei at puberty (Table 1), whereas the ER concentration in

Laboratory of Endocrinology, Faculty of Biology, M. V. Lomonosov Moscow University.
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TABLE 1. ER Level in Uterine Cells and Hepatocytes of Female Rats Differing in Their Endocrine Status (M ± m)

Groups of animals	CR, moles/mg protein × 10^{-14}	Bonds per cell	P<	NR, moles/mg DNA × 10^{-14}	Bonds per cell	P<	Total number of bonds per cell	P<
Sexually mature: uterus (5)	15,7±3,0	15 070±1 935		30,8±8,0	1233±323		16 303±1 956	
liver (10)	2,0±0,26	2 145±294	0,001	6,7±0,6	266±24	0,02	2 467±313	0,001
Ovariectomized (18)	3,4±0,3	3 392±314	0,01*	7,8±0,63	311±25		3 540±298	0,02*
Sexually immature (3)	0,21±0,01	206±12	0,001**	0,90±0,01	36±5	0,001**	242±10	0,001**
Hypophysectomized (5)	0,97±0,16	809±137	0,02**	2,1±0,7	84±31	0,001**	1 012±147	0,02**
Hypophysectomized +200 μg STH for 5 days (5)	2,9±0,8	2 634±732	0,05***	2,1±0,6	82±24		2 718±738	0,05***

Legend. Number of determinations given in parentheses. Where not indicated in which organ ER was determined, data given are results of study of ER in hepatocytes. 1, 2, and 3 asterisks denote significance of differences between parameters compared with mature, ovariectomized, and hypophysectomized animals respectively.

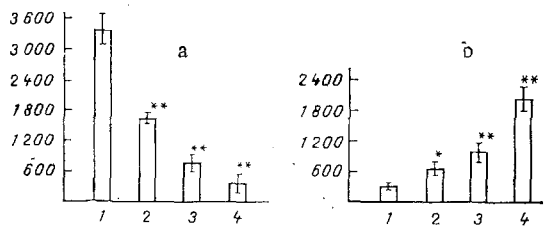

Fig. 1. Effect of injection of various doses of E_2 on ER level in cytosol (a) and nuclei (b) of liver cells of ovariectomized female rats (M ± m). Ordinate, content of CR and NR (in bonds per cell). 1) Control group of ovariectomized female rats 1 h after injection of 0.2 ml propylene glycol (n = 18); 2, 3, 4) 1 h after a single injection of 1 μg (n = 3), 25 μg (n = 3), and 500 μg (n = 8) E_2 respectively. *P < 0.05, **P < 0.001 compared with control.

the uterus falls at puberty [3, 4]. Another point to note is that 3 days after ovariectomy the ER content in the liver cytoplasm increased significantly, while it remained unchanged in the nuclei. In the uterus, however, there was a marked reduction in their number under these circumstances [9].

The strong dependence of ER concentration on pituitary function will be noted. For instance, in hypophysectomized animals the content of both CR and NR in the cell was appreciably less than in intact mature females, whereas E_2 reception in the uterus evidently is not so highly dependent on the pituitary [3].

The presence of different mechanisms controlling the ER content in different organs (liver and uterus) can be postulated. In all probability the ER concentration in cells of the uterus depends largely on the circulating estrogen level in the blood, whereas this factor does not play a decisive role for the liver. This conclusion is confirmed also by "fluctuation" of the ER content in the cytosol and nuclei of uterine cells depending on the stage of the cycle [6, 7] and its absence in hepatocytes [13].

The existence of a certain pituitary hepatotrophic factor (HF; "feminotrophin"), which participates in realization of the action of sex hormones on the liver [8, 10], has recently been put forward. "Feminotrophin" is most probably one hormone or a complex of already known pituitary hormones, among which the most realistic candidate for this role is STH [8].

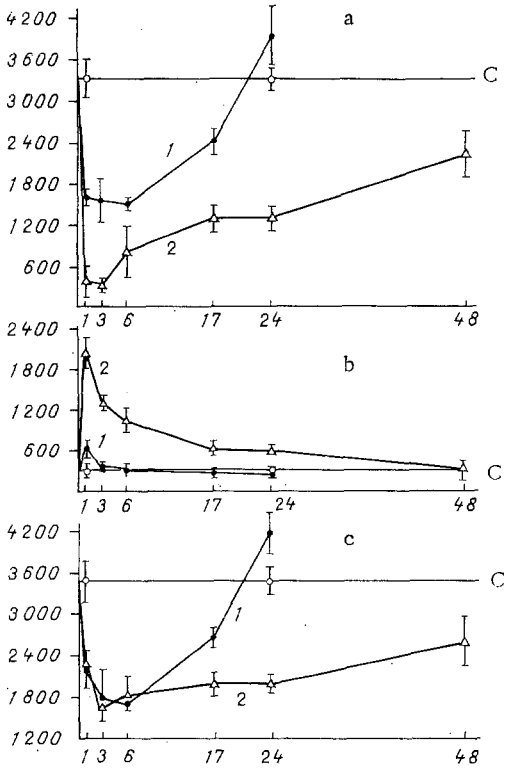

Fig. 2. Time course of content and intracellular distribution of ER in the liver of ovariectomized rats after a single injection of 1 μg (1) or 500 μg (2) of E_2 (M ± m). Abscissa, time after injection of E_2 (in h); ordinate, ER content in cytosol (a) and nuclei (b) of hepatocytes and their total content in cell (c) (in bonds per cell). C) Control animals after injection of 0.2 ml of solvent. Each point on graph is mean of three to five determinations.

To test this hypothesis, STH was injected (200 μg daily for 5 days) into hypophysectomized animals and an increase in their ER content was observed up to a level almost indistinguishable from normal (Table 1).

Our data thus confirm the view that pituitary factors participate in regulation of E_2 reception by hepatocytes through their action on the ER content in the cell.

If the hypothesis on the existence of a pituitary HF is accepted and it is assumed that its secretion depends in turn on the sex steroid levels in the body, the following hypothesis can be expressed: The long lasting fall in the E_2 level arising after ovariectomy leads to an increase in secretion of pituitary HF and, as a result of this, to the increase in ER content which we observed in the cytosol fraction of hepatocytes after ovariectomy.

Investigations showed that single injections of various (1, 25, and 500 μg) doses of E_2 into ovariectomized female rats caused interconnected dose-dependent changes in the ER level in the cell cytosol and nucleus (Fig. 1). A fall in the CR level (Fig. 1a) and an increase in the NR content (Fig. 1b) took place 1 h after injection of the hormone. The degree of decrease in the number of receptors in the cytosol and its increase in the nucleus rose with an increase in hormone concentration. This reciprocal redistribution of ER between cytosol and nuclear cell fractions under the influence of the injected hormone is characteristic of the "classical" target organs for steroid hormones [5] and, as is generally considered, is evidence of translocation of steroid hormone complexes from receptors into the nucleus [11].

The presence of a phenomenon of translocation of estrogen-receptor complexes in the nucleus under the influence of injection of E_2 *in vivo* may be an important argument in support of inclusion of the liver among the target organs for estrogens.

The characteristic dose-dependent redistribution of ER between cell cytoplasm and nucleus was confirmed by a study of the time course of the CR and NR content after injection

of E_2 (Fig. 2). The two extreme doses of E_2 were investigated: 1 and 500 μg. The results showed that 1 h after injection there was a decrease in the CR content (Fig. 2a), which was more marked after a dose of 500 μg, accompanied by an increase in the NR content (Fig. 2b). A dose of 1 μg caused only a twofold increase in NR ($P < 0.05$), whereas a dose of 500 μg increased the ER content in the nucleus almost sevenfold ($P < 0.001$). The increase in NR after a dose of 500 μg lasted a considerable time, until 24 h after the injection ($P < 0.01$), whereas 3 h after injection of 1 μg E_2, NR was back to the control level. A dose-dependent dynamics of CR also was observed: 17-24 h after injection of 1 μg, CR reached the control level, whereas injection of 500 μg caused long-lasting "depression" of the CR content, which still remained below the control level even 48 h after injection.

The dose-dependent dynamics of the total ER content in the hepatocytes is shown in Fig. 2c. In the course of 1-3 h after injection of both doses a "deficit" of the ER content was observed in the hepatocytes, which disappeared 17-24 h after injection of a small dose of E_2, but which was preserved for a long time after injection of 500 μg of E_2. The phenomenon of a "deficit" in the total ER content in the target cells after injection of the hormone *in vivo* has been found by many other investigators in various organs, notably the uterus [5, 12], and they have explained it by temporary elimination of only nuclear [5] or of both nuclear and cytosol [14] receptors in the course of the receptor cycle. Under these circumstances all investigators have noted that the degree of the receptor deficit depends on the dose of hormone and that the most intensive elimination of receptors occurs during the first few hours after injection of the hormone, as was the case in the present experiments also.

Despite the essential qualitative similarity between the processes of dynamic redistribution of ER in hepatic and uterine cells under the influence of injection of E_2 *in vivo*, organ-specific differences also were observed. For instance, effects comparable in the degree of ER translocation into the nucleus are produced in the uterus and liver by different doses of estrogens injected *in vivo*. To achieve submaximal translocation (60-70%) of ER into hepatocyte nuclei a dose two orders of magnitude higher is required than for the uterus [5-7]. This phenomenon, in all probability, can be explained by elevation of the threshold of sensitivity of the liver cells to the action of E_2, which is due to the lower ER concentration in the hepatocytes coupled with rapid metabolism of hormones in this organ, and also, probably, lower sensitivity of chromatin to E_2-receptor complexes.

Reception of E_2 by liver cells thus obeys the general rules of reception of hormones by target cells, and also possesses some essential organ-specific differences.

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