MULTICOMPONENT SYSTEM OF ESTROGEN-BINDING PROTEINS IN THE LIVER CYTOSOL: SPECIES DIFFERENCES IN RATS, MICE, AND GUINEA PIGS

A. N. Smirnov, O. V. Smirnova,
N. K. Shul'gina, and V. B. Rozen

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Estradiol(E_2)-binding proteins of the liver cytosol of sexually mature female and male rats, mice, guinea pigs, and rabbits were investigated by gel-filtration on Sepharose 6B columns. Components with Stokes' radii (*a*) of 6-7 and 2.5 nm, forming specific, stable complexes with E_2, were found in the liver of female rats and of male and female mice. Components with values of (*a*) of 6-7, 2.5, and under 2.0 nm, specifically binding E_2, were discovered in the liver of male rats and male and female guinea pigs. Complexes of E_2 with components with (*a*) values of 6-7 and under 2.0 nm were relatively stable, whereas the complex of E_2 with the component with $a = 2.5$ nm could dissociate completely in 45 min. It is suggested that at least some of the components of these heterogeneous populations of E_2-binding proteins may participate in the reception of the estrogen signal by the liver. KEY WORDS: estrogen-binding proteins; liver; species differences.

In the modern view a central place in the mechanism of action of steroid hormones and, in particular, of estrogens is occupied by the stage of interaction of the hormones with intracellular receptor proteins [5]. Specific estrogen-binding proteins, possessing some of the properties characteristic of receptors, were found previously in the cytosol and nuclei of rat liver cells [1, 4, 6, 10]. Fractionation of the cytoplasmic fraction of the liver showed, however, that the population of these proteins is heterogeneous: in rats there are at least four estrogen-binding components which differ in size and in the kinetic parameters of interaction with estradiol (E_2) [2, 3]. Well-marked sex differences in the representation of these components also were found in the animals; the differences discovered are partly determined by the function of the gonads [3].

In this investigation an attempt was made to answer the following questions: 1) are specific estrogen-binding proteins present in the liver not only of rats, but also of animals of other species; 2) is the heterogeneity of the E_2-binding proteins of the liver a feature confined to rats or is it more general in character; 3) are the sex differences in E_2-binding proteins in the liver found in rats characteristic of members of other species of rodents also? The answers to these questions, in the writer's opinion, could make a definite contribution to the discovery of the ways whereby these hormonal influences mediated by estrogen-binding proteins are realized in the liver.

EXPERIMENTAL METHOD

Sexually mature male and female rats, mice, guinea pigs and, in some experiments, rabbits of mixed populations were used. All the principal stages of the work were described previously [3]. A brief description of the methods used is given below. The liver was perfused with cold 0.9% NaCl solution and homogenized in 10 mM Tris-HCl buffer (pH 7.5) containing 1.5 mM EDTA, 10 mM KCl, and 6 mM dithiothreitol. The homogenate was centrifuged at 105,000 g for 1 h. To determine the total binding of E_2 aliquots of the supernatant fraction (20-30 mg protein/ml) or blood serum in a volume of 1 ml were incubated for 1.5 h with 1-2 ng of 17β-estradiol-6,7-^{3}H (E_2-^{3}H) with a specific radioactivity of 44 Ci/mmole. To determine nonspecific binding, together with E_2-^{3}H, 400 ng of unlabeled E_2 also was added. In the experiments to study the kinetics of dissociation of hormone-protein complexes the cytosol was incubated with E_2-^{3}H for 45 min, after which the incubation continued for a further 45 min in the presence of 400 ng of unlabeled E_2. At the end of incubation samples were applied to a column (1.6 × 30 cm) of Sepharose 6B, previously calibrated against standard pro-

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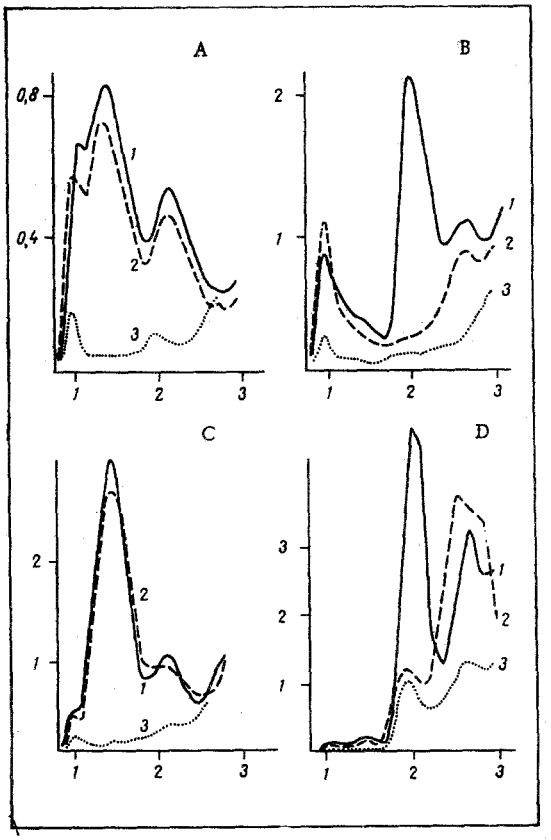

Fig. 1. Elution profiles of estradiol-^{3}H with proteins of liver cytosol of different species of rodents from Sepharose 6B columns. A) Female rats; B) male rats; C) male mice; D) female guinea pigs. 1) Cytosol incubated with E_2-^{3}H only for 1.5 h; 2) cytosol incubated for 45 min with E_2-^{3}H, excess unlabeled E_2 added, and incubation continued for a further 45 min; 3) cytosol incubated simultaneously with E_2-^{3}H and excess of unlabeled E_2 for 1.5 h. Abscissa, ratio of elution volume (V_e) and the free volume of the column (V_0): ordinate, radioactivity (in cpm/ml · 10^{-3}). The results given in C and D are analogous to those obtained for individuals of the opposite sex.

teins. For elution (0.3 ml/min) the same buffer was used as for homogenization, with the exception of the dithiothreitol. The content of radioactivity was determined in the fractions of eluate. Each experiment was repeated three to five times.

EXPERIMENTAL RESULTS

Elution profiles of complexes of E_2-^{3}H with cytoplasmic liver proteins of the three species of rodents from Sepharose 6B columns are illustrated in Fig. 1. Clearly in all cases peaks of E_2-^{3}H bound by proteins were discovered and these were partly or completely suppressed by an excess of unlabeled hormone. It could accordingly be concluded that these proteins have a limited capacity for E_2 and, in all probability, have relatively high affinity. In the liver cytosol of animals of all three species there is not one, but two or three (not counting heavy aggregates, excluded from the column) specific E_2-binding components, which differ in size. A specific E_2-binding component with a Stokes' radius (a) of 6-7 nm ($V_e/V_0 \simeq 1.4$) was discovered in both female and male rats, mice, guinea pigs, and also rabbits. This component was easily saturated with hormone (Fig. 1, curve 3) and bound it firmly (Fig. 1, curve 2). In rats considerable sex differences were found in the

occurrence of the other specific estrogen-binding proteins (Fig. 1A, B). Meanwhile in mice and guinea pigs no such sex differences were found. On the other hand, substantial species differences occurred in the content of these proteins. The systems of estrogen-binding proteins can usefully be divided into two types depending on the composition of the lower-molecular-weight forms ($a \leq 2.5$ nm). The first type – the female rate type – also was characteristic of male and female mice. The distinguishing feature of this type of system was that it contained, besides protein with a = 6-7 nm, only one other E_2-binding component with $a \simeq 2.5$ nm ($V_e/V_0 \simeq 2.0$). Proteins of this type, as well as the component with a = 6-7 nm, are readily saturated with hormone and bind it firmly (Fig. 1A, C). The other essential feature is that the binding capacity of the component with a = 6-7 nm was greater than the capacity of the component with $a \simeq 2.5$ nm. The second type of system of estrogen-binding protein – the male rat type – also was found in the liver cytosol of male and female guinea pigs. This type was characterized by the presence of two (not counting the component with a = 6-7 nm) components specifically binding E_2, with $a \simeq 2.5$ nm and under 2.0 nm ($V_e/V_0 \simeq 1.6$).* Both components were saturated with E_2. However, the component with $a \simeq 2.5$ nm, by contrast with the protein with $a < 2.0$ nm and the other estrogen-binding liver proteins, formed a labile bond with the hormone (Fig. 1). It should also be noted that the binding capacity of the component with $a \simeq 2.5$ nm was considerably greater than the capacity of the component with a = 6-7 nm, especially in guinea pigs.

To study whether the E_2-binding components found in the liver cytosol are the result of contamination of the cell proteins by impurities from the blood, experiments were carried out along similar lines but using blood serum from the same species of animals. In these experiments no specific binding of E_2-^3H by the blood serum proteins of any of the groups of animals was found. In every case only nonspecific binding of E_2-^3H by proteins with $a \simeq 3.0$ nm, not suppressed by an excess of unlabeled hormone, was discovered. In all probability, this binding was due to complex formation between E_2-^3H and albumin. In addition, in the blood serum of male and female guinea pigs nonspecific binding of the hormone by a component with $a < 2.0$ nm was found. The proteins specifically binding E_2 detected in the liver cytosol of the animals studied are thus cell proteins and not analogs in the blood serum.

It can be concluded from these results that the heterogeneity of the E_2-binding components in the liver cytosol is a relatively universal rule. The question of the functional role of these components remains unanswered. It can be tentatively suggested that at least some of the E_2-binding proteins perform the role of mediators in the action of estrogens on the liver, i.e., that they are receptors for these hormones. The main claimant for this role is the component with a = 6-7 nm, detected in the liver cytosol of all the groups of animals studied. This protein is evidently analogous to the 8S-receptors of estrogens in target organs [5]. It may therefore be expected that this component mediates those hormonal effects that are most common to all sensitive tissues. The results provide additional information on the possible role of the E_2-binding component of male rats with $a \simeq 2.5$ nm, which the writers previously [3] called "special estrogen-binding protein" (SEBP). Roy et al. [7, 8] found a parallel in the changes in α_{2u}-globulin in rat urine, which is synthesized in the liver, and the androgen-estrogen-binding protein of the liver (probably identical with SEBP) during individual development and in the course of various experimental procedures. These workers concluded from their observations that the hormone-binding protein is a receptor for steroids, mediating their action on α_{2u}-globulin synthesis. The absence of any analogous estrogen-binding protein in the liver of mice, in which sex-dependent excretion of proteins has also been shown to take place [9], throws doubts on the validity of the above hypothesis. A more likely cause, in the writers' view, of the above-mentioned parallel trend in the changes in these two proteins is the similarity (or identity) of the mechanism of their regulation. As regards the functional role of other estrogen-binding components of the liver, information at present available is insufficient to allow any firm suggestions. Further investigations are evidently required into the problem of species and sex differences in the action of estrogens on the liver.

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*It was impossible to determine the value of a for this component on the Sepharose 6B columns more accurately; the possibility cannot be ruled out that the small value of a could be only apparent on account of adsorption of the protein by the gel.

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INSULIN-FORMING ACTIVITY OF MONOLAYER CULTURES OF BOVINE FETAL PANCREATIC ISLET CELLS

V. P. Fedotov, V. N. Blyumkin, R. A. Babikova, N. V. Sadovnikova, V. V. Abramova, and B. I. Shal'nev

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The insulin concentration in the growth medium of primary monolayer cultures of bovine fetal pancreatic islet cells grown in the presence of a normal and increased (300 mg%) glucose content, was determined by a radioimmunologic method. A high glucose concentration led to increased secretion of insulin. The results of the cytological study showed definite correlation between mitotic activity of the cells of the culture and the intensity of insulin secretion into the medium. KEY WORDS: monolayer culture; islet cells; insulin secretion; mitotic activity.

A method of obtaining and growing monolayer primary cultures of bovine fetal pancreatic islet cells was described previously [1, 2]. It was shown that islets and more extensive areas of monolayer, consisting of epithelial cells, a high proportion of which contain aldehyde-fuchsin-positive granules in their cytoplasm, are formed 3-4 days after seeding of the cells. The addition of an increased concentration of glucose (final concentration 300 mg%) to the culture medium led to degranulation of the cells after 24 h. These results indicate that the cells described above correspond in their morphological characteristics to the B-cells of the islets of Langerhans.

This paper describes the results of determination of the insulin-forming activity of cultures of bovine fetal pancreatic islet cells at different times of culture and under particular experimental conditions.

EXPERIMENTAL METHOD

Monolayer cultures of islet cells were obtained from the pancreas of 4-6-month bovine fetuses with the aid of solutions of collalytin and trypsin [1, 2]. Cultures were grown in Carrel's dishes and flasks of various sizes, and also in penicillin flasks with coverslips. The medium was changed 2-3 days after seeding. The insulin concentration in the culture medium was determined by a radioimmunologic method using kits from the firm CEA-IRE-Sorin. The limit of sensitivity of the method in these experiments was 5 microunits/ml medium. The cultures of islet cells were fixed at appropriate times of the experiment with 96°C ethanol, stained with hematoxylin-eosin or aldehyde-fuchsin, and examined cytologically.

EXPERIMENTAL RESULTS

A. Hormonal Activity of the Bovine Fetal Pancreatic Cell Cultures. In the first stage of the work mixed monolayer cultures were obtained in which the contribution of the B-cells did not exceed 8-10%. In such cultures from the very beginning there were many fibroblasts, proliferation of which inhibited proliferation of the cells by the 10th-15th day after seeding. On the 3rd-5th day of culture (1-3 days after changing the medium 199 with a normal glucose concentration for the same fresh medium) the insulin concentration in the culture

Laboratory of Biological Standardization of Hormones, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Pathomorphology, Institute of Transplantation of Organs and Tissues, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 8, pp. 235-238, August, 1978. Original article submitted July 28, 1977.

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