including the liver has been discussed in the literature [1, 12]. Moreover, an increase in the rate of incorporation of ^{14}C-leucine into low density lipoproteins has been demonstrated on a model of alloxan diabetes [14]. Participation of insulin in the mechanisms of formation of hyperlipoproteinemia induced by a high carbohydrate diet will thus be evident.

LITERATURE CITED

1. S. A. Morenkova, Byull. Éksp. Biol. Med., No. 4, 419 (1978).
2. J. E. H. Ahrens, J. Hirsch, and K. Cette, Trans. Ass. Am. Phys., 74, 134 (1961).
3. L. A. Carlson, J. Atheroscler. Res., 3, 334 (1963).
4. R. P. Eaton and D. H. Kipnis, Am. J. Physiol., 217, 1168 (1969).
5. R. P. Eaton, R. Dase, and D. S. Schade, Metabolism, 25, 245 (1976).
6. S. Eisenberg and D. Rachnilewitz, Biochim. Biophys. Acta, 326, 391 (1973).
7. J. Folch, N. Lees, and G. H. S. Stanley, J. Biol. Chem., 226, 497 (1957).
8. S. R. Ford, Am. J. Clin. Nutr., 21, 904 (1961).
9. C. I. Glueck, Diabetes, 18, 739 (1968).
10. R. I. Havel and H. A. Eder, J. Clin. Invest., 34, 1345 (1955).
11. S. Koga, L. Bolis, and A. N. Scanu, Biochim. Biophys. Acta, 236, 416 (1971).
12. A. Korner, J. Endocrinol., 20, 256 (1960).
13. R. U. Manley, T. P. Bersot, and V. S. Le Quire, Science, 168, 380 (1970).
14. A. C. Nestruck and D. Rubinstein, Can. J. Biochem., 54, 617 (1976).
15. E. J. Schaefer, S. H. Eisenberg, and R. I. Levy, J. Lipid Res., 19, 367 (1978).

TRANSLOCATION OF DIFFERENT TYPES OF LIVER ESTROGEN RECEPTORS FROM CYTOPLASM TO NUCLEUS

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One of the most important properties of classical steroid hormone receptors is their ability to be translocated, in the form of a complex with the hormone after activation which is dependent on temperature and various other factors, from the cytoplasm of the cell into the nucleus – the main site of action of the hormone [10].

The aim of the present investigation was to determine whether different components of the heterogenous population of estrogen receptors (ER) found in the cytoplasm of the liver in male and female rats [1, 4, 5], possess this property, and also, should it be found that different forms of ER can be translocated, to study the basic principles of this process from the comparative point of view. The cytosol estradiol (E_2) receptor of the rat uterus, in which, as the writers showed previously [1], one form of liver ER is essentially similar in many of its physicochemical and hormone-binding parameters, was used for this comparative analysis. The tasks undertaken in the investigation are one approach to the solution of the problem of the functional role and significance of the existence of different forms of ER in the liver for the realization of the many different effects of estradiol both within the same tissue and also in different tissues.

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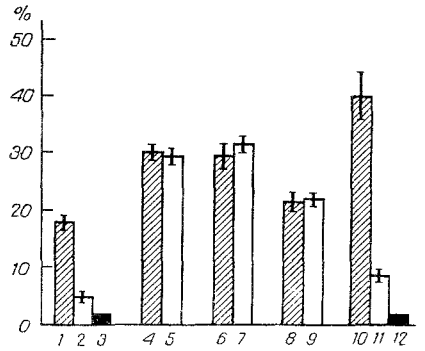

Fig. 1

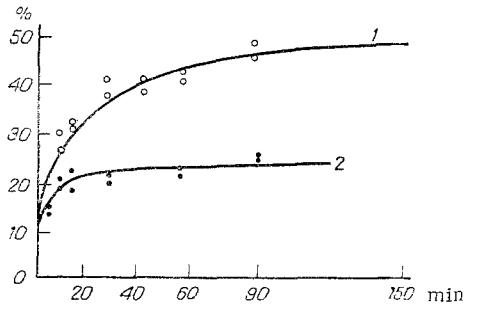

Fig. 2

Fig. 1. Effect of temperature processing and gel filtration on level of translocation of specific ERC into nuclei. 1, 4, 6, 8, 10) ERC preincubated for 30 min at 25°C; 2, 5, 7, 9, 11) without preincubation at 25°C; 3, 12) specific binding of free ^3H-E_2 dissolved in buffer. 1-9) Translocation into liver nuclei of specific complexes of ^3H-E_2: with receptors of whole female liver cytosol (1-3), with type I ER of male (4, 5) and female (6, 7) liver, and with type II ER of female liver (8, 9). 10-12) Translocation into uterine nuclei of ERC from whole uterine cytoplasm. Type I ER of male liver cytosol and types I and II ER of female liver cytosol obtained by gel filtration on AcA-44 ultragel [1]. ERC obtained by preincubation of hepatic or uterine receptors with 400 pg/ml of ^3H-E_2 in the presence (nonspecific binding) or absence (total binding) of excess of unlabeled E_2 for 2 h at 0-4°C and incubated with a suspension of hepatic or uterine nuclei at 0-4°C for 1.5 h. Specific binding of ERC with nuclei determined from difference between total and nonspecific binding and expressed as a ratio of specific binding in cytosol added to nuclei, which was determined by adsorption on dextran-coated charcoal [1]. Here and in Figs. 2 and 3 results of one of three experiments are shown. Ordinate, ratio of ERC bound by nuclei to added ERC (in %).

Fig. 2. Kinetics of translocation into hepatic nuclei of specific complexes of ^3H-E_2 with types I (1) and II (2) ER from female rat liver. Types I and II liver ER were obtained by preliminary treatment of the cytosol with ammonium sulfate at 40% saturation and subsequent gel filtration on AcA-44 ultragel [1, 4]. Otherwise the experimental scheme was the same as in Fig. 1. Abscissa, time (in min); ordinate, ratio of ERC bound with nuclei to added ERC (in %).

EXPERIMENTAL METHOD

Sexually mature male and female rats of a mixed population were used. The cytosol was prepared and the various forms of liver ER were isolated and partially purified by precipitation with ammonium sulfate, gel filtration, and ion-exchange chromatography as described previously [1, 4].

In medium of low ionic strength two forms of ER were discovered by gel filtration of female liver cytosol on Sepharose 6B: a high-molecular-weight form I (sedimentation constant $K_S = 8S$, Stokes' radius 6-7 nm), similar in dimensions and properties to the E_2 receptor of uterine cytosol, and a smaller form II with $K_S = 4S$ and Stokes' radius of about 3.0 nm [1]. Under the same conditions a type I ER common to males and females and a so-called special estrogen-binding protein (SEBP) with $K_S = 3.8S$ and Stokes' radius of 2.5 nm, present only in males and possessing some properties distinguishing it from other ER [1, 4], were discovered in the liver cytosol of males.

Translocation of estradiol-receptor complexes (ERC) into the cell nuclei of liver and uterus was studied in a cell-free system in vitro at 0-4°C. Purified hepatic and uterine nuclei were obtained by the method in [7] with modifications. To obtain ERC the test material was incubated with [2,4,6,7-^3H]-E_2 (specific radioactivity 90-100 Ci/mmole) as described previously [1]. The quantity of hepatic and uterine ERC translocated into the nuclei was estimated from the appearance of radioactivity in the nuclear residue and from the decrease in the content of specific ERC in the cytosol. The translocation level was estimated as the ratio between specific ERC bound with the nuclei and added ERC.

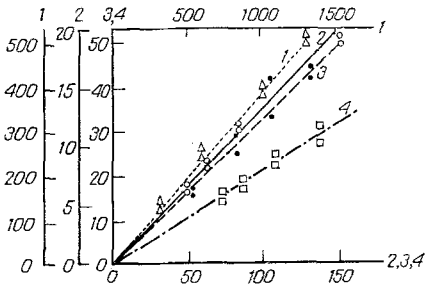

Fig. 3. Binding with nuclei of ^3H-E_2 complexes with high-molecular-weight cytosol ER from rat uterus (1) and from male (2) and female (3) liver and with type II ER from female rat liver cytosol (4) with different ratios between cytosol ERC and nuclear DNA. Ratio of cytosol ERC to nuclear DNA was varied as follow: To a standard quantity of nuclear suspension a definite volume of cytosol, preincubated with increasing amounts of added ^3H-E_2, was added. Otherwise the experimental scheme was the same as that described in Fig. 1. Abscissa, concentration of added ERC (in pmoles/mg DNA); ordinate, quantity of added ERC (in pmoles/mg DNA).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that ERC of the whole unfractionated liver cytosol of female rats (1-3), ^3H-E_2 complexes with type I ER of male (4, 5) and female (6, 7) liver, and also with type II ER from female liver (8, 9) have the ability to be transported into liver nuclei in a similar manner to the translocation of ERC from the cytosol into the nuclei of the uterus (10-12). However, in the case of ERC from whole hepatic and uterine rat cytosol an essential condition for effective translocation into the nuclei of these organs is preliminary activation of the ERC by a raised temperature, whereas ^3H-E_2 complexes with different structural forms of liver ER, separated from each other by gel filtration, possess increased affinity for nuclei even without temperature processing, i.e., they are already preactivated. The data obtained on the activating effect of factors of different nature (raised temperature, gel filtration) can be regarded as confirmation of the hypothesis that a low-molecular-weight inhibitor of activation is present in the cytosol of hormone-competent organs [9, 11].

Comparative analysis of the kinetics of translocation of high-molecular-weight ERC from male and female rat liver and from rat uterus showed that the process is essentially similar in different tissues. Absence of any marked degree of organ-specificity of the translocation process will be noted, as regards both receptor protein and the source of the nuclei: Uterine ERC moved into liver nuclei in the same way as high-molecular-weight liver ERC. Replacement of hepatic nuclei by uterine nuclei likewise did not affect the results.

Comparison of the kinetics of translocation of complexes of E_2 with types I and II ER from female liver into the cell nuclei showed certain differences. For instance, the maximal level of translocation was reached sooner in the case of type II than in the case of type I (Fig. 2). The maximal level of translocation of form I under these circumstances was twice as high as that of type II ER. To determine whether these differences in the level of translocation are connected with different degrees of aggregation of these types of liver ER, translocation of the above-mentioned ERC into the nucleus was studied in a medium with increased ionic strength (0.3 M NaCl), which causes reversible dissociation of the 8S form into 4S subunits, similar in size to type II ER [5]. Under these conditions more marked inhibition of translocation of type I into the nuclei was observed, as a result of which the level of translocation of the two forms of female liver ER became the same. In all probability the existence of a receptor in the aggregated 8S form somehow facilitates interaction of activated ERC with chromatin, which leads to the more effective binding of type I ER than of type II, which cannot form aggregates, in the nuclei.

To study the character of interaction of E_2 receptors with the acceptor structures of the nuclei, translocation of complexes of E_2 with types I and II liver ER and with the uterine receptor was studied with different

relative proportions of interacting components. The results are evidence (Fig. 3) that even when very large quantities of ERC were added, 2 or 3 orders of magnitude higher than the possible ratios between ERC and nuclear DNA in the cell in vivo, saturation of the nuclear acceptor sites was not found in any single case, in agreement with observations by most other investigators [2, 6, 8].

It must be emphasized that the unsaturability of the nuclear ERC binding sites discovered by these experiments and those of other workers cannot be regarded as proof of the nonspecificity of interaction of ERC with chromatin. There is perhaps a limited number of specifically binding sites in chromatin, which can be masked by the high level of nonspecific binding of ERC necessary for their accumulation in the nuclei [3, 12]. Selectivity of interaction of ERC with nuclei may also be connected with the pre-existing ability of different genes to react in different ways to addition of the hormone–receptor complex [3].

The third type of liver ER investigated, namely SEBP, which has a number of unique binding properties [4], also differs from other liver ER in its behavior in the cell, for experiments in vitro did not reveal translocation of complexes of E_2 with SEBP into liver nuclei. The physiological role played by SEBP in the cell [4] perhaps does not require its translocation into the nucleus.

The study of interaction between complexes of E_2 with various types of liver ER and cell nuclei thus showed the essential similarity of these processes for high-molecular-weight ERC of male and female rat liver and rat uterus, as well as definite differences in the general principles of translocation of ERC from cytosol into nucleus in a medium with low ionic strength for types I and II liver ER and, in particular, for complexes of E_2 with SEBP, which are not translocated into the nucleus. The facts described above attach great credibility to the hypothesis that different forms of liver ER participate in the realization of the many different effects of E_2 in that organ. It can be tentatively suggested that besides the common mechanisms of E_2 reception in different tissues, unique pathways for initiation of the effects of estrogens also exist in the liver.

LITERATURE CITED

1. G. D. Mataradze, M. Kh. Rukaiya, A. N. Smirnov, et al., Biokhimiya, 44, 1484 (1979).
2. N. A. Romanova, G. A. Romanov, and B. F. Vanyushin, Dokl. Akad. Nauk SSSR, 243, 1585 (1978).
3. N. A. Romanova, G. A. Romanov, V. B. Rozen, et al., Biokhimiya, 44, 529 (1979).
4. A. N. Smirnov, O. V. Smirnova, and V. B. Rozen, Biokhimiya, 42, 560 (1977).
5. O. V. Smirnova, A. N. Smirnov, U. M. Fisher, et al., Dokl. Akad. Nauk SSSR, 226, 474 (1976).
6. M. Atger and E. Milgrom, Biochim. Biophys. Acta, 539, 41 (1978).
7. J. Chauveau, J. Moule, and C. Rouiller, Exp. Cell Res., 11, 317 (1956).
8. G. C. Chamness, A. W. Jenning, and W. L. McGuire, Biochemistry (Washington), 13, 327 (1974).
9. A. Goidl, M. H. Cake, K. P. Dolan, et al., Biochemistry (Washington), 16, 2125 (1977).
10. E. V. Jensen, S. Mohla, T. Gorell, et al., J. Steroid Biochem., 3, 445 (1972).
11. B. Sato, J. Nichizawa, K. Noma, et al., Endocrinology, 104, 1474 (1979).
12. K. Jamamoto and B. Alberts, Annu. Rev. Biochem., 45, 721 (1976).