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ESTRADIOL RECEPTORS IN HUMAN LIVER

M. J. DUFFY and G. J. DUFFY
Department of Nuclear Medicine, St. Vincent's Hospital, Dublin 4, and
Departments of Medicine and Surgery, University College, Dublin 4, Ireland

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SUMMARY

High affinity, low capacity estradiol binding activity was detected in cytoplasmic fractions from human liver. The binding activity was clearly differentiated from that due to contaminating serum proteins. Binding was specific for estrogens and there was no evidence of specifically bound androgen or progestin. Heating the cytoplasmic fraction or treatment with proteolytic enzymes destroyed activity, suggesting that the binding component was a protein. It is concluded that human liver contains a specific estradiol receptor with steroid specificity similar to that found in other estrogen target tissues. The presence of the estradiol receptor may be responsible for action of estradiol in modulating the synthesis of specific proteins in liver.

INTRODUCTION

It is now generally accepted that one of the primary steps of estradiol action is combination of the steroid with cytoplasmic protein molecule termed the estradiol receptor [1, 2]. Such estradiol receptors (ER) are found in a number of mammalian tissues including the uterus, pituitary, hypothalamus, [1, 3] breast tumors [4] and were recently detected in liver from the rat, mouse, rabbit and green monkey [5, 6]. Although estradiol stimulates the synthesis of a number of specific proteins in human liver, [7] studies on the mode of action of the steroid in this organ have not to date been reported. In this paper we describe the presence of an estradiol binding protein in human liver and show that its binding specificity is similar to that of the estradiol receptor previously reported for the rat uterus.

MATERIALS AND METHODS

Human liver was obtained at post-mortem from postmenopausal female subjects. [2,4,6,7,-*n*-^{3}H]-estradiol (96 Ci/mmol) was purchased from the Radiochemical Center. R5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione), [^{3}H]-R5020 (56.5 Ci/mmol), R1881 (methyltrienolone) and 6,7-[^{3}H]-R1881 (55.5 Ci/mmol) were gifts from Dr. J. P. Raynaud, Roussel-Uclaf and C1-628 was a gift from Parke Davis & Co. Other chemicals were supplied by Sigma London Ltd.

Receptor assay using dextran-coated charcoal. Cytoplasmic fractions from liver were prepared as previously described [8, 9].

The estradiol receptor was assayed by the method of Duffy and Duffy[9] except that incubation was carried out at 4°C for 20 h. Nonspecific binding was measured in the presence of 100 fold excess diethyl stilbestrol (DES). Androgen receptors were measured using the synthetic androgen R1881 as ligand [10] and the progesterone receptor using the synthetic progestin R5020 [11]. Nonspecific binding was determined in the presence of 500 fold excess unlabelled synthetic steroid. Incubation was also for 20 h at 4°C For each receptor the free steroid was separated from protein-bound steroid using dextran-coated charcoal [9].

Receptor assay using protamine sulfate. The cytosol fractions were prepared identically as for the dextran-charcoal assay. The incubation was carried out as described by Chamness *et al.*[12]. 0.25 ml of protamine sulfate (1 mg/ml) was added to 0.1 ml of liver supernatant fraction. The mixture was vortexed, allowed to stand at 0°C for 5 min and then centrifuged at 2000 *g* for 10 min. The supernatant was aspirated and [^{3}H]-estradiol (concentration range 5.10^{-8}–5.10^{-9} M) was added to the precipitate. Nonspecific binding was measured in the presence of 100 fold excess DES. Incubation was again carried out at 4°C for 20 h. The precipitate was washed and the protein bound estradiol was extracted as previously described [12].

Determination of protein concentration. Protein was measured as previously described by Lowry *et al.*[13].

RESULTS

In four different experiments in the present investigation the concentration of ER varied between 5 and 27 fmol receptor/mg of cytoplasmic protein. Similar values were obtained when using either dextran-charcoal or protamine sulfate to separate free from receptor bound estradiol. Using the synthetic androgen, R1881 and progestin, R5020 as ligands, no receptor for these steroids was detected in any of the liver cytoplasmic fractions.

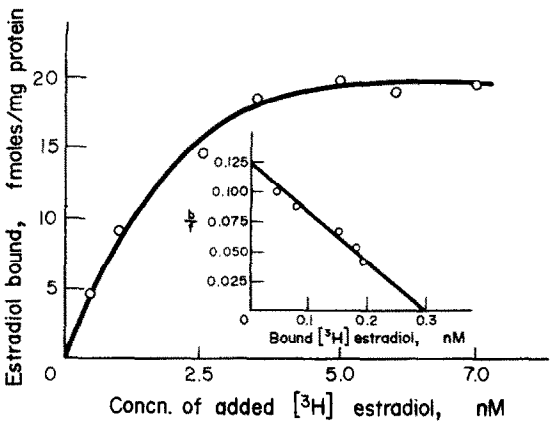

Fig. 1. Effect of adding increasing concentrations of [^{3}H]-estradiol on the amount of estradiol bound by human liver supernatant. Procedure as described in section in Methods. Dextran-coated charcoal was used to separate free from bound [^{3}H]-estradiol. Inset Scatchard plot of data in curve.

Saturation of ER. Figure 1 shows the effect of adding increasing concentration of [^{3}H]-estradiol on the amount of steroid bound. Saturation of receptor is reached at a concentration of 2–5 nM added estradiol concentration. When the curve in Fig. 1 is plotted as described by Scatchard[14] a straight line is obtained, (Fig. 1 inset), suggesting a single class of binding sites over the concentration range of steroid used. The concentration of binding sites determined from the intercept of the abscissa was 0.296 nM in this liver cytosol and the apparent K_D from the slope of the graph was 2.4×10^{-9} M.

Specificity of the ER. The data in Table 1 show that the ER is highly specific for estradiol. Excess unlabelled (100×) estradiol, estriol, DES and the antiestrogen C1-628 significantly reduced the binding of the [^{3}H]-estradiol. On the other hand the nonestrogenic steroids testosterone, progesterone and cortisol had no significant effect on [^{3}H]-estradiol binding.

Stability of the ER. Heating the cytoplasmic fraction at 48°C for 60 min completely abolished the specific binding. Neither the proteinase inhibitor aprotinin (100 K.I.U.), or the reducing agents dithiothreitol (0.5 mM) or mercaptoethanol (0.5 mM) significantly increased the measured concentration of the receptor. Treatment of the extract with trypsin (0.1 mg) and chymotrypsin (0.1 mg) reduced binding by 79 and 73% respectively whereas ribonuclease (0.1 mg) or deoxyribonuclease (0.1 mg) were without significant effect.

Table 1. Effect of unlabelled steroids and antiestrogens on [^{3}H]-estradiol binding by human liver cytoplasm

Competing steroid added	[^{3}H]-estradiol binding (% of control)
None	100
Estradiol	11.3
Estriol	25.6
DES	12.7
C1-628	18.3
Testosterone	95.6
Progesterone	90.2
Cortisol	102.0

Cytoplasmic fractions were incubated with 5×10^{-8} M estradiol in the presence and absence of 100 fold excess unlabelled compound as described in section on Methods. Dextran-charcoal was used to separate free from bound estradiol. Values are means of 3 separate experiments each carried out in duplicate.

Differentiation of ER binding from that due to serum proteins. Since a number of plasma proteins, especially albumin [15] and sex hormone binding globulin (SHBG) [8], are capable of binding estradiol, and the liver is the probable site of synthesis of these proteins it was important to establish that estradiol was not bound by these non-receptor proteins.

Evidence suggesting that serum proteins were not involved in specific binding is as follows;

(i) When using either dextran-charcoal or protamine sulfate, binding was inhibited by excess DES a compound which is not thought to bind with high affinity to SHBG. On the other hand excess unlabelled testosterone which binds tightly to this serum protein had no significant effect on estradiol binding.

(ii) Using protamine sulphate there was no specific binding of either [^{3}H]-testosterone or dihydrotestosterone. This finding indicates that SHBG was not precipitated by the protamine and was thus unlikely to have contributed to the estradiol binding.

(iii) The specific binding was completely destroyed by incubating the cytoplasmic fraction at 48°C for 60 min a criterion used to differentiate the labile receptor from the relatively heat stable binding due to plasma proteins [16].

(iv) The low K_D obtained (Fig. 1) for the estradiol protein interaction suggests albumin was not involved in specific binding in the present investigation.

DISCUSSION

It is known that estradiol increases the synthesis of a number of plasma proteins both in animals and in man. These proteins are probably formed in the liver and include thyroxine-binding globulin, renin substrate, pre-β-lipoproteins, transcortin and some specific proteins involved in blood-clotting [7]. Eisenfeld *et al.*[5] have suggested that the estradiol induced synthesis of some of these proteins may be responsible for the side effects of estrogen-containing oral contraceptives. In spite of these many actions of the steroid on liver, this tissue is not normally regarded as a target organ for estradiol action.

The present investigation shows that human liver contains a high affinity, low capacity binding protein specific for estradiol. A similar protein has recently been detected in liver from a number of animal species including the rat and the green monkey [5, 6]. These proteins may be responsible for mediating the above mentioned effects in liver. The binding protein

in human liver described here exhibits steroid specificity similar to that previously described for the ER in rat liver [5], uteri [1] and breast tumors [8].

The results obtained in this investigation may explain why the presence of ER in liver metastases from human breast tumors were unreliable in predicting response to hormonal therapy [17]. If the normal liver already contained ER the mere presence of the receptor in metastases might not be sufficient for predicting response to endocrine therapy. It may, therefore, be necessary for accurate prediction to have a critical minimum concentration of receptors in these metastatic tumors as has recently been shown for ER values in the primary breast tumor [18].

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