NOTE

Cytoplasmic Estrogen Receptor in Castrated Rat Thymus

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Abstract

Characterization of estrogen-binding components was attempted in cytosol fractions from thymus, spleen and mesenteric lymph node of castrated rats. As shown by sucrose gradient analysis, specific binding of [6, 7-3H]estradiol-17β in the thymus is associated with a component migrating at 4 S. The binding of [6, 7-3H]estradiol-17β is highly specific since it is easily displaced by unlabeled estradiol-17β and diethylstilbestrol. Affinity of unlabeled estrone, estriol and clomiphene citrate, is much lower, and estradiol-17α, progesterone, testosterone, 5α-dihydrotestosterone and corticosterone have no affinity for the component at all. The dissociation constant of thymic estrogen binding is 0.25 nm in males and 0.3 nm in females. The number of binding sites is 6 fmols/mg protein in both sexes. No specific binding to estrogen is, however, found in cytosol fractions from the other two tissues.

Enzyme- and heat-experiments demonstrate that specific estrogen binder in thymic cytosol is heat-labile and at least protein in nature. It is concluded that the rat thymus contains a cytoplasmic estrogen receptor which is in part protein and heat-labile.

Estrogen, like adrenocortical hormones, regularly induces acute involution of the thymus in intact animals (Dougherty, 1952; Thompson et al., 1965; Thompson et al., 1966), but its effect on lymphoid tissues other than the thymus is minimal and quite variable. According to Thompson et al. (1965), and Thompson et al. (1966), this involution is due to total loss of the cortex and reduced cellularity in the shrunken medullary area of estrogen-treated mouse thymus, which is characterized by diminished nucleic acid content and deoxyribonucleic acid synthesis within the tissue. In the review by Dougherty (1952), it is mentioned that estrogen exerts its moderating action on the thymus by continuously destroying lymphocytes. Estrogen administered to newborn mice, like thymectomy, induces wasting syndrome characterized by both central and peripheral lymphoid hypoplastasia, weight loss, diarrhea and increased motality (Thompson and Russe, 1965). This phenomenon is at present understood as a result of the suppression of immune response and the inability to resist infection. Moreover, estrogen suppresses the incorporation of [3H]thymidine by phytohemagglutinin-stimulated blood lymphocytes (Ablin et al., 1974). These reports clearly suggest that the effect of estrogen is on the development and maintenance of lymphoid tissues and the immune system.

According to the current view of the mechanism of action of steroid hormones, such phenomena should be mediated by spe-
cific intracellular hormone receptors (O'Malley and Means, 1974; Liao, 1975; O'Malley and Schrader, 1976). Recently, Seiki et al. (1978) showed the high uptake and long-term retention of estrogen by mouse lymphoid tissues. They also presented data indicating the presence of cytoplasmic estrogen receptor in mouse thymus (1979). The present study was, in turn, designed to characterize cytoplasmic estrogen-binding macromolecules in the rat lymphoid tissues and to compare differences between the properties of binders in rat and those in mouse.

Materials and Methods

Chemicals

[6,7-3H]Estradiol-17β ([3H]E2β, 50 Ci/mmoll was obtained from New England Nuclear Corporation, Boston; estrone (E1), estradiol-17α (E2α), estradiol-17β (E2β), estriol (E3), progesterone (P), testosterone (T), 5α-dihydrotestosterone (5α-DHT) and corticosterone (CC) from Sigma Chemical Company, St. Louis; diethylstilbestrol (DS) from Kyorin Pharmaceutical Company, Tokyo; protease from Kaken Chemical Company, Ltd., Tokyo; ribonuclease-A (bovine pancreas origin, Type 1-A), bovine serum albumin (BSA) and human gamma globulin (H1G, Cohn fraction II) from Sigma Chemical Company; dextran T 70 from Pharmacia Fine Chemicals, Uppsala, Sweden; and charcoal (Norit A) from American Norit Company, Florida. Clomiphen citrate (CL) was a gift from Shionogi Pharmaceutical Company, Osaka.

Cytosol preparation

Male and female rats of DA strain, seven weeks old and 180-200 g body wt, were castrated. Four days after the operation they were decapitated, and the thymus, spleen and mesenteric lymph node were excised. The tissues were rinsed in an ice-cold TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.4) to remove adhering blood and connective tissues. Cytosol fractions were prepared as previously described (Seiki et al., 1979), and the cytosol protein concentration was measured by the method of Lowry et al. (1951).

Sucrose gradient analysis and binding specificity

Cytosols from the thymus, spleen and lymph node of both sexes were incubated at 4°C for 3 hr in 1 nM [3H]E2β plus a 100-fold molar excess of various non-labeled steroids. Pilot studies indicated that 1 nM [3H]E2β produced saturation binding to the cytosols, and this concentration was selected for the gradient assay. A 0.3 ml aliquot of each incubate was proceeded to sucrose density gradient ultracentrifugation and dextran-coated charcoal adsorption as described previously (Seiki et al., 1979). Steroid specificity of binding was determined by comparing the amounts of bound radioactivity extracted from each sample after the density gradient assay.

Binding kinetics

Affinity and capacity of estrogen-binding components in thymic cytosols from both sexes were examined by adding 0.3 ml of the cytosol to tubes containing varying concentrations of [3H]E2β or [3H]E2α plus a 100-fold excess of unlabeled E2β. The tubes were incubated for 3 hr at 4°C, and 0.7 ml of dextran-coated charcoal suspension was added. After shaking and standing for 10 min, charcoal was sedimented by 800~g centrifugation for 10 min. The data were analysed according to Scatchard (1949) after subtracting non-specific binding calculated from the preparations incubated with the non-labeled hormone.

Steroid analysis

Thymic cytosols from both sexes incubated with 1 nM [3H]E2β at 4°C for 3 hr were applied to the density gradient assay as described above. After collecting the fractions containing 4S binding components, the composition of steroids in the components was analysed by thin-layer chromatography as described previously (Seiki et al., 1979).

Results and Comments

Sedimentation patterns of cytosols from the lymphoid tissues of male rats are shown in Fig. 1. In the thymic cytosol, an appreciable amount of radioactivity migrates with a component in the 4S region. A 100-fold molar excess of unlabeled E2β displaces the 4S peak in the thymus. Cytosols from the spleen and lymph node similarly have a radioactivity peak in the region which is not displaced by an excess of non-labeled E2β. Cytosols from the same tissues of females also show similar sedimentation patterns to those in males (Fig. not shown). Binding specificity of cytosols from the lymphoid tissues was determined by com-
Fig. 1. Sedimentation pattern of cytosols from lymphoid tissues of castrated male rats incubated with 1 nM [3H]E2 for 3 hr at 4°C. Following adjustment to equal protein concentration (10 mg/ml), samples of 0.3 ml were layered on 5-20% sucrose gradients. Free steroids were removed by dextran-coated charcoal adsorption before analysis. Protein concentration in each fraction (0.15 ml) was monitored by measurement of the absorbance at 280 nm.

Table 1. Steroid specificity of cytoplasmic estrogen binding components in castrated rat lymphoid tissues

<table>
<thead>
<tr>
<th>100-fold excess of unlabeled steroid</th>
<th>Specifically bound [3H]E2 (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymus</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>E1</td>
<td>71.5</td>
</tr>
<tr>
<td>E2α</td>
<td>94.4</td>
</tr>
<tr>
<td>E2β</td>
<td>24.1</td>
</tr>
<tr>
<td>E3</td>
<td>69.8</td>
</tr>
<tr>
<td>DS</td>
<td>24.3</td>
</tr>
<tr>
<td>CL</td>
<td>73.2</td>
</tr>
<tr>
<td>P</td>
<td>98.5</td>
</tr>
<tr>
<td>CC</td>
<td>96.6</td>
</tr>
<tr>
<td>T</td>
<td>96.6</td>
</tr>
<tr>
<td>5α-DHT</td>
<td>97.6</td>
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</table>

Cytosols were incubated with 1 nM [3H]E2 alone (control) or with a 100-fold molar excess of unlabeled steroids. Following sucrose gradient centrifugation and dextran-coated charcoal adsorption, the amount of bound radioactivity was collected from fractions 6 to 13 designated in Figure 1.

petitive binding (Table 1). In thymic cytosols, E2β and DS have very high affinity for the binder. The affinities of E1, E3, and CL are low, whereas the remaining steroids used do not compete with [3H]E2β. In cytosols from the lymph node and spleen, none of the steroid hormones tested competes with [3H]E2β. These findings reveal that there is an estrogen receptor in the 4 S region only in the thymic cytosol but not in the other two tissues, which is in good agreement with our recent data reported for the lymphoid tissues of mice (Seiki et al., 1979). In the present experiment, however, unlabeled E2α did not compete with [3H]E2β for the 4 S binder in the thymic cytosol, but in the previous study (Seiki et al., 1979) using mouse thymus this hormone showed partial competition with the radiolabeled E2β. The reason for this difference is not
Fig. 2. Scatchard plots of $[^{3}H]E_2\beta$ binding in thymic cytosols from castrated male (○) and female (▲) rats. Samples of 0.3 ml were incubated with 0.5–100 nm $[^{3}H]E_2\beta$ with or without a 100-fold molar excess of unlabeled $E_2\beta$. Specific binding was measured by dextran-coated charcoal adsorption as described under Methods. Each point is the mean of assay duplicates. The values are adjusted to correspond to a protein concentration of 1 mg/ml. B: amount bound, U: amount unbound.

yet clear but it is probable that the difference depends on the species and ages of animals, the dosage of steroid hormones and incubation conditions, etc.

The Scatchard plots (Fig. 2) show that $[^{3}H]E_2\beta$ binds specifically to estrogen binding components in thymic cytosols with a dissociation constant of 0.25 nM in males and 0.3 nM in females, and a number of binding sites of 6 fmols/mg protein in both sexes. Binding affinity of the order of 0.1 nM is very high, being almost equivalent to cytoplasmic estrogen-receptors in the mouse thymus (Seiki et al., 1979), the uterus of various species (Toft et al., 1967; Notides, 1970; Giannopoulos and Gorski, 1971), the rat hypothalamus (Korach and Muldoon, 1974; Kato, 1975), the rat pituitary (McGuire et al., 1973), the rat adrenal (Cutler et al., 1978) and the rat ovary (Saiduddin and Zassenhaus, 1977). Binding capacity of the order of 1 fmols/mg protein is very low, being almost equivalent to cytoplasmic estrogen receptors in mouse thymus (Seiki et al., 1979), guinea pig brain tissue (Pasqualini et al., 1978) and rat adrenal (Cutler et al., 1978), but higher than that in rat pituitary (McGuire et al., 1973), rat hypothalamus (Kato, 1973; Kato, 1975) or rat ovary (Saiduddin and Zassenhaus, 1977). These data reveal that the 4 S component in rat thymus is of high affinity-low capacity to bind estrogen, which is quite similar to the 4 S estrogen receptor in mouse thymus (Seiki et al., 1979).

Thymic cytosols from both sexes pre-incubated with $[^{3}H]E_2\beta$ were treated with 200 µg protease or 30 µg ribonuclease for 30 min at room temperature, or heated at 60°C for 5 min. It was found that protease completely inhibited the binding of radioactivity, whereas ribonuclease was without significant effect. The binding was further eliminated by heating the cytosols. These properties of the binding component mean that the component is in part protein and heat-labile.

Steroid analysis (Table 2) of the 4 S binding components in the thymic cytosols labeled with $[^{3}H]E_2\beta$ shows that over 60% of the radioactivity is of unaltered $E_2\beta$. This is in agreement with our previous findings on mouse thymus (Seiki et al., 1979).

The presence of cytoplasmic estrogen receptor in the thymus of the mouse (Seiki et al., 1979) and the rat (present study) has particular bearing on the action of this hormone on the thymus through its receptor.
Table 2. Steroid analysis by thin-layer chromatography of 4 S binding component in cytosols from castrated rat thymus incubated with 1 nM [3H]E2β

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% Radioactivity</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>9.1</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>E2β</td>
<td>67.2</td>
<td>62.1</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>12.3</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>11.4</td>
<td>16.2</td>
<td></td>
</tr>
</tbody>
</table>

* Details are given under methods.

Acknowledgement

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References