ISOLATION OF AN ESTRADIOL-BINDING SYSTEM FROM THE GUINEA PIG UTERUS

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An estradiol-binding system not previously described has been isolated from the uterus of sexually immature, sexually mature, and pregnant guinea pigs. The association constant of the system relative to estradiol was determined and the characteristics of the specificity of binding and stability are given. The results suggest that the system thus isolated can be used in methods to determine estradiol based on competitive binding of hormones by proteins.

The most promising methods of determining microdoses of steroid hormones at the present time are those based on competitive binding of hormones with proteins (CBP). In particular, to determine estrogens, the receptor proteins of the uterus are used. Binding systems of this type have been isolated from the uterus of various species of animals (rabbit, rat, mink, etc.) and man [1, 3, 5-9]. However, only the system from the rabbit uterus has been applied for practical purposes [2, 4], for it possesses greater specificity of binding than the others. However, even this system is not sufficiently stable and, in addition, animals in a particular physiological state must be used for its isolation (on the 6th day of pregnancy) [2]. Accordingly the evaluation of the properties of the estradiol-binding systems of the uterus in animals not so far investigated and tests of the applicability of these systems to the CBP method are of considerable interest.

The isolation of an estradiol-binding system from the guinea pig uterus is described in this paper and the comparative characteristics of the system are given.

EXPERIMENTAL METHOD

Experiments were carried out on female guinea pigs of three physiological groups: 1) sexually immature weighing 105 g, 2) sexually mature weighing 300 g, and 3) pregnant, weighing 500 g. The animals were decapitated and the uterus was removed and washed with cold 0.25 M sucrose solution and weighed. The tissue was cut into small pieces in the cold with scissors and homogenized in 10 volumes of 0.25 M sucrose containing 10 mM Tris−HCl, pH 8.0, and 1 mM EDTA, for 10 min in a glass homogenizer. Homogenization continued for 10 sec (at 60 rpm), with an interval of 20 sec. The homogenate was centrifuged at 4°C on a Spinco L2 ultracentrifuge at 105,000 g for 60 min or at 800 g for 15 min. The supernatants obtained in this way were used for determination of binding power. For this purpose, increasing quantities (from 50 to 300 pg) of 6, 7-estradiol-17β-H^3 (specific activity 20 Ci/mmole, Radiochemical Centre, Amersham, England) in ethanol were added to the tubes. The ethanol was evaporated to dryness. The samples were then treated with 0.1 ml Tris−HCl buffer (0.01 M, pH 8.0) and 0.1 ml of the supernatant. Incubation was carried out for 30 min at 30°C. The sorbent used to remove the free steroid was a 0.35% suspension of activated charcoal (OU, mark B) in 0.0025% dextran solution (mol. wt. 80,000) in a volume of 0.5 ml. The steroid adsorbed on the charcoal was removed by centrifugation, and samples of 0.4 ml were taken from the supernatant for determination of the radioactivity. The samples were treated with PPO/POPOP/dioxan scintillator and radioactivity counted on a type SBS-1 counter (efficiency with respect to H^3 25%). When standard displacement curves were plotted, together with labeled estradiol in a saturating concentration, increasing quantities of

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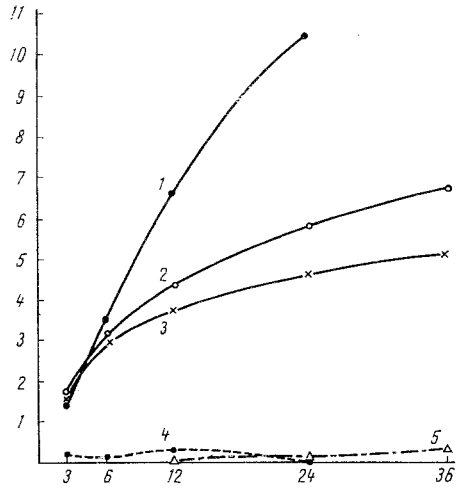

Fig. 1

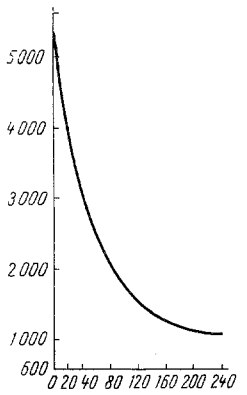

Fig. 2

Fig. 1. Characteristics of binding power of receptors of the guinea pig uterus: 1) sexually immature guinea pigs; 2) sexually mature guinea pigs; 3) pregnant guinea pigs; 4) heated binding system; 5) albumin. Abscissa, quantity of labeled estradiol added (in $\frac{\text{pulses}}{100\ \text{sec}} \times 10^3$); ordinate, quantity of hormone bound with protein (in $\frac{\text{pulses}}{100\ \text{sec}} \times 10^3$).

Fig. 2. Standard curve of displacement of estradiol-H^3 by its unlabeled analogue. Abscissa, quantity of unlabeled estradiol added (in pg); ordinate, radioactivity of protein-bound labeled hormone (in pulses).

the unlabeled hormone were added to the system. In that case, during incubation the labeled and unlabeled estradiol became distributed between the bound and free fractions.

EXPERIMENTAL RESULTS

The first stage of the investigation was to determine the characteristics of the binding power of the isolating system. Curves of saturation of the system with increasing quantities of labeled estradiol were plotted (Fig. 1). A system of the uterus of sexually immature guinea pigs (the maximal percentage of binding of added hormone was 87.5) possessed the greatest binding power. The binding power of the systems of sexually mature and pregnant animals was less: 37.5 and 31%, respectively. The association constants of the isolated systems with estradiol were determined at the same time, using the method suggested by Meŝter et al. [7]. The nonlinearity of the resulting Scatchard curves indicates that the system tested contains binding systems of high and low specificity. The values of the binding power calculated from the curves are 0.013-0.090, 0.013-0.041, and 0.013-0.028 pmole/mg wet weight of uterine tissue of the sexually immature, sexually mature, and pregnant guinea pigs, respectively. The association constants for the same groups of animals were 1.6×10^{10}, 3.3×10^{10}, and 6×10^{10} M^{-1}, respectively. These values are in agreement with those in the literature obtained on other species of animals [1, 7, 10].

The second stage of the investigation was to study the specificity of binding of sex hormones by the isolated binding system. Specificity was assessed by the ability of the hormones to displace labeled estradiol from the complex with the binding system and was expressed as a percentage. The displacing power of each steroid tested was determined for the concentrations of 40 and 160 pg. A standard displacement curve of estradiol by its unlabeled analogue is given in Fig. 2. The experiments showed that androsterone and testosterone have the least affinity for the binding system (below 5%). Estrone and estriol were bound to a greater degree (by 27 and 42%, respectively). The specificity of binding was confirmed by incubation of the supernatant with increasing quantities of labeled estrone and testosterone. It was also shown that replacement of the binding system by albumin led to the total disappearance of binding (Fig. 1).

The stability of the binding system was assessed from the change in binding power when the supernatant was kept for a week at −20°C. Under these circumstances there was a gradual decrease in the binding

power and a decrease in slope of the displacement curve. Similar relationships were observed for binding systems of the rabbit uterus [3]. Treatment of the supernatant for 30 min at 60°C led to total inactivation of the system (Fig. 1).

The fact will be noted that if two supernatants (obtained at 105,000 g and at 800 g) were used, the resulting patterns were completely analogous. There is reason to suppose that the binding of estradiol in both cases was achieved by the same binding system.

Because of the high binding power, the specificity, and the simplicity of isolation of the estradiol-binding system obtained from the guinea pig uterus described above, it can be used as a method of determining microdoses of estrogens.

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