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CHARACTERISTICS OF THE ESTROGEN RECEPTOR SYSTEM OF THE GUINEA PIG UTERUS

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The physicochemical parameters of estradiol–receptor (E_2–R) interaction in the cytosol of guinea pig uterus (the velocity constants of association and dissocation, the half-life of the E_2–R complex, the change in free binding energy) were studied and the content of receptors in the cell calculated. With different degrees of approximation to a state of equilibrium, for most steroids the percentage of affinity for the test system remained unchanged, indicating that equilibrium had been reached in these cases. The affinity of the steroids for the R system analyzed was determined by integrity of the 3- and 17 β-hydroxyl groups and by the somewhat greater importance of the third phenolic hydroxyl group of the steroid molecule.

KEY WORDS: uterus; steroid–receptor interaction; specificity; estrogens.

This investigation is a continuation of a study of the estradiol–receptor (E_2–R) system of the guinea pig uterus started previously [1].

Kinetic and thermodynamic parameters and also the specificity of interaction of a number of steroids with the receptor system of the uterus were studied.

EXPERIMENTAL METHOD

Uteri of sexually immature guinea pigs weighing 140-200 g were removed and treated by the usual method [1] with dilution of the tissue with buffer 1:10 for homogenization. The cytosol was kept at −30°C. The residue was used for DNA determination [3]. 2,4,6,7-Estradiol-17β-^{3}H (E_2-17β-^{3}H) (specific activity 100 Ci/mmole, Amersham) and unlabeled compounds, whose names are given in Table 1, were used in the experiments. The velocity constants of dissocation (k_{-1}) and association (k_{+1}) and the half-life of the complex ($T_{1/2}$) were determined by the usual methods [8]. To determine the equilibrium association constant (K_{as}) of estradiol with the isolated system the principle of saturation analysis was used [4]. The range of concentrations of labeled estradiol was 0.05-0.75 pM. The free and bound fractions were separated by means of a suspension of charcoal (0.25% suspension in buffer containing 0.1% gelatin). To allow for nonspecific binding, a hundred-fold excess of unlabeled estradiol was used [5]. K_{as} and the concentrations of binding sites were calculated by Scatchard's

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TABLE 1. Nomenclature of the Compounds Tested

1) 3,4-bis-(p-hydroxyphenyl)hexene-3
2) D-estratriene-1,3,5(10)-diol-3,17β
3) 3,4-bis-(p-hydroxyphenyl)hexadiene-2,4
4) D-17α-ethinyl-estratriene-1,3,5(10)-diol-3,17β
5) D-3-hydroxyestratriene-1,3,5(10)-one-17
6) D-3-benzoylhydroxyestratriene-1,3,5(10)-ol-17β
7) D-17β-valeroylhydroxyestratriene-1,3,5(10)-ol-3
8) D-estratriene-1,3,5(10)-triol-3,16α,17β
9) D-4-bromoestratriene-1,3,5(10)-diol-3,17β
10) D-2-bromoestratriene-1,3,5(10)-diol-3,17β
11) D-estratriene-1,3,5(10)-ol-3
12) L-estratriene-1,3,5(10)-diol-3,17β
13) D-estratriene-1,3,5(10)-ol-17β
14) D-estratriene-1,3,5(10)-one-17
15) D-2-formylestratetraene-1,3,5(10),9(11)-diol-3,17β
16) D-2-oximinomethylestratetraene-1,3,5(10),9(11)-diol-3,17β
17) D-pyrazolo[3,2-c]estratetraene-1,3,5(10),9(11)-ol-17β
18) D-2-methyleneestradiene-4,9-ol-17β-one-3
19) D-estradiene-4,9-diol-3,17β
20) D-2α-methylestradiene-4,9-diol-3,17β
21) D-2-spiro-(Δ'-pyrazolino-3)-estradiene-4,9-ol-17β-one-3
22) D-2-spirocyclopropanoestradiene-4,9-ol-17β-one-3
23) Acid oxalate of D-2α-dimethylaminomethylestradiene-4,9-ol-17β-one-3
24) D-3-L-oximinoestradiene-4,9-ol-17β
25) D-3-estratriene-1,3,5(10)-oximinoestradiene-4,9-ol-17β
26) D-2-cyanomethylestradiene-4,9-ol-17β-one-3
27) D-3-L-oximino-2-methyleneestradiene-4,9-ol-17β
28) D-estradiene-4,9-ol-17β-one-3
29) D-17β-acetoxyestradiene-4,9-one-3
30) D-6-chloro-17α-acetoxy-1α,2α-methylene-pregnandiene-4,6-dione-3,20
31) D-17α-acetoxy-2-methylenepregnene-4-dione-3,20
32) DL-estratriene-1,3,5(10)-diol-3,17β
33) D-estrone-5(10)-diol-3,17β
34) D-5α-adrostanol-3α-one-17β
35) D-5-adrostanol-3β-one-17
36) 2-hydroxyphenanthrene
37) p-hydroxyazobenzene
38) DL-D-homoestrapentaene-1,3,5(10),6,8-ol-3-one-17α
39) DL-14β-D-homoestrapentaene-1,3,5(10),6,8-ol-3-one-17α

method [10]. The change in free binding energy (ΔG) of estradiol (E_2) with the receptor (R) was calculated from the second law of thermodynamics $\Delta G = \Delta H - T\Delta S$ and, as a consequence of it, $\Delta G = -RT \ln K_{as}$. The relative affinity of the test steroids was determined by the method described previously [2], with linearizing of the standard curve in logarithmic coordinates.

EXPERIMENTAL RESULTS AND DISCUSSION

During analysis of the breakdown of the E_2-R complex of the cytosol of the guinea pig uterus on incubation with excess of unlabeled E_2 at 30°C, fast and slowly dissociating components of the reaction mixture were found, and Table 2 gives the velocity constants of dissocation (k_{-1}) and the half-life of the E_2–protein complex ($T_{1/2}$). The fast-dissociating component was a nonspecific complex. The parameters of dissociation of this complex, as determined in the present experiments, were the same as those given in the literature [8]. To test that dissociation of the E_2-R complex (formed at 30°C) on temperature, its behavior was analyzed at 0°C. No appreciable dissociation was observed under these conditions.

Preliminary experiments [1] showed that equilibrium in the E_2-R system of the cytosol of the guinea pig uterus was reached after 30 min. The stability of the E_2-R complex at equilibrium lasted for 5 h.

In the present investigation the thermodynamic parameters of E_2-R interaction were studied during incubation for 5 h at 30°C. The values of the association constants (K_{as}), changes in the free binding energy in the reaction of E_2-R interaction (ΔG), and data on the number of binding sites calculated per cell (Q) are given in Table 2.

The values obtained for K_{as} at 30°C differed by an order of magnitude from the same values for the rabbit, rat, and human uterus [7, 8]. Data in the literature on the E_2-R concentration of the cell are analogous to those given in Table 2 [9]. The change in the free binding energy, calculated from the experimental values of K_{as} point to a determining role of hydrogen bonds in the formation of the E_2-R complex of the uterine cytosol, was in full agreement with the view expressed in the literature on steroid–protein interaction [6].

When the affinity of various compounds with a known velocity of interaction with the R system was studied under close to equilibrium conditions, the maximal allowable incubation time (because of the stability of the complex) was chosen to be 5 h.

The characteristics of affinity of 39 compounds (the nomenclature of these compounds is given in Table 2) are shown in Table 3; 14 of them were studied previously under different conditions of incubation, namely 30 min [2], and 25 compounds were chosen for the first time. The characteristics of affinity are expressed as molar percentage relative to the affinity of E_2, and as values of the equilibrium association constants for the reaction of interaction between the test steroids and the receptor system of the uterus.

Comparison of the results of incubation for 30 min and 5 h points to the absence of any significant changes in the percentage affinity of most steroids. Essential changes were observed for estradiol 17β-valerianate and 17α-ethinylestradiol, for 2-and 4-Br-estradiols and, probably, in the case of the 17β-valerianate they can probably be explained by metabolic conversion of this steroid during prolonged incubation, whereas for the other compounds they can be explained by the ratio between the parameters of the kinetics of formation of receptor complexes with estradiol and the above-mentioned preparations. Among the nonsteroid compounds, a lengthening of the incubation period in principle increased the percentage affinity of diethylstilbestrol, so that a more careful analysis of the kinetics of the binding of this compound is required.

The percentage affinity of several compounds (2,3,5,6,8,11-14 – see Table 3) showed no significant change when incubation was prolonged from 30 min to 5 h, evidence of the presence of equilibrium in the E_2-S-R systems (S represents the test compound), and justifies the calculation of K_{as} in these cases only.

On the basis of these results certain views expressed previously on the rules governing the formation of the steroid–receptor complex in the cytosol of the guinea pig uterus can be widened. An essential condition for active interaction between the steroid molecule and receptor is integrity of the third phenolic and 17β-hydroxyl groups; these hydroxyl groups, moreover, are unequal in importance, for if the hydroxyl group is removed from the phenolic position the activity of the R complex thus formed is only half that of the compound formed by removal of the 17th hydroxyl group (compound No. 11, 13). Removal of the hydroxyl group in position 3 and simultaneous oxidation of that in position 17 reduces the activity of E_2-R interaction still more (compound No. 14).

Modification of the steroid molecules in positions 17 and 3 also reduces the activity of complex formation. The results of this investigation revealed that the degree of this decrease depends in a definite manner on the character of the modification. For instance, removal of the OH group in position 17 (compound No. 11) reduces the activity of complex formation much more than oxidation or esterification with valeric acid. Removal of the OH group in position 3 sharply reduces S–R interaction (compound No. 13), whereas esterification with benzoic acid (compound No. 6) reduces this interaction much less.

Additional information was obtained on the effect of the immediate surroundings of the principal functional groups on binding. For instance, introduction of an ethinyl group in the 17α position only slightly reduced E–R interaction (compound No. 4), whereas introduction of a hydroxyl group in position 16α (compound No. 8) and bromination in position 4 (compound No. 9) changed the affinity of the steroid by an order of magnitude. Bromination in position 2 (compound No. 10) had an even greater effect on the phenolic hydroxyl group.

Modification of the steroid skeleton after simultaneous introduction of substituents in position 2 and with integrity of the 3- and 17β-hydroxyl groups sharply reduced binding (compound No. 15).

The mirror isomer of estradiol (compound No. 12) binds with the receptor by two orders of magnitude less strongly than its biologically active analog.

TABLE 2. Physicochemical Characteristics of Estradiol Receptor System of Cytosol of Guinea Pig Uterus (M ± m)

Component	k_{-1}, sec^{-1} n=8	$T_{1/2}$, min	K_{as}, M^{-1} n=7	K_{+1}, $sec^{-1} \cdot M^{-1}$	ΔG, kJ/mole	Q, thousands of sites per cell, n=5
Specific	$(0{,}112 \pm 0{,}005) \times 10^{-3}$	102	$(0{,}390 \pm 0{,}053) \times 10^{9}$	$0{,}437 \times 10^{6}$	—50	36±3
Nonspecific	$(0{,}165 \pm 0{,}003) \times 10^{-2}$	7	—	—	—	—

Legend. 1. In each experiment tissue from 50 animals was used. 2. Values of $T_{1/2}$, k_{+1}, and ΔG were calculated from the corresponding mean values of k_{-1} and K_{as}.

TABLE 3. Characteristics of Affinity of Certain Compounds for Estradiol-Receptor System of Cytosol of Guinea Pig Uterus (M ± m)

No.	Compound	Incubation for 30 min, molar % of affinity	Incubation for 5 h, molar % of affinity	K_{as}, M^{-1}
1	Diethylstilbestrol	54,19±6,84	202,63±11,25	—
2	Estradiol	100	100	$(0{,}390 \pm 0{,}053) \times 10^{9}$
3	Dihydrostilbestrol	70,55±3,94	69,81±5,51	$(0{,}265 \pm 0{,}023) \times 10^{9}$
4	17 α-Ethinylestradiol	88,05±2,17	66,13±3,61	—
5	Estrone	24,59±4,25	18,38±1,72	$(0{,}536 \pm 0{,}045) \times 18^{8}$
6	Estradiol 3-benzoate	22,63±2,23	22,03±1,86	$(0{,}765 \pm 0{,}067) \times 10^{8}$
7	Estradiol 17β-valerianate	7,91±1.01	17.19±1,56	—
8	Estriol	9,96±2,34	8,06±0,83	$(0{,}223 \pm 0{,}018) \times 10^{8}$
9	4-Br-estradiol	2,62±0,44	4,90±0,55	—
10	2-Br-estradiol	1,29±0,00	3,14±0,56	—
11	17-Deoxyestradiol	1,47±0.23	1,38±0,11	$(0{,}481 \pm 0{,}028) \times 10^{7}$
12	L-Estradiol	1,35±0,10	1,03±0,36	$(0{,}300 \pm 0{,}080) \times 10^{7}$
13	3-Deoxyestrone	0,44±0,06	0,62±0,11	$(0{,}208 \pm 0{,}037) \times 10^{7}$
14	3-Deoxyestrone	0.19±0.09	0.15±0,01	$(0{,}511 \pm 0{,}048) \times 10^{6}$
15—31 and 33—39	See Table 1	—	<0,15	—
32	D,L-estradiol	—	44,0±10,2	—

Among the nonsteroid compounds, the unique properties of the diethylstilbestrol molecule will be noted, for among all the compounds investigated it had the highest affinity for the R system of the guinea pig uterus.*

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