CHARACTERISTICS OF THE GUINEA PIG OVIDUCT ESTROGEN RECEPTOR SYSTEM

N. D. Fanchenko, R. N. Shchedrina,
L. S. Minina, and E. A. Novikov

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The kinetic and thermodynamic parameters of estradiol-receptor (E_2-R) interaction in the cytosol of guinea pig oviducts were investigated. Specificity of interaction of 14 compounds with the test system was characterized. The R-system of the cytosol of the guinea pig oviduct was shown to have high affinity for E_2 and limited capacity. The change in the free binding energy of 52 kJ/mole is evidence of the stability of the resulting E_2-R complex. Activity of formation of the steroid-receptor (S-R) complex is attributable to integrity of the hydroxyl groups in positions 3 (phenolic) and 17β of the steroid molecule, as shown by changes in the degree of S-R interaction after different types of modifications of the steroids in various positions. S-R interaction is also affected by the immediate environment of the hydroxyl groups in positions 3 and 17. The properties of the nonsteroid estrogen diethylstilbestrol are noteworthy.

KEY WORDS: oviducts; kinetics and thermodynamics; specificity of estrogen reception.

There is a wealth of evidence in the literature on the properties of the estradiol receptor (E_2-R) systems of a number of target organs [4, 6, 7, 12, 13]. However, it is difficult to compare these data because of differences in the species of animals and the methods used, although such a comparison is extremely necessary for the understanding of the mechanism of regulation of the reproductive function and the search for ways of goal-directed synthesis of substances with a selective action on a particular stage of the reproductive system.

The object of this investigation was to study the kinetic and thermodynamic parameters determining E_2-R interaction in guinea pig oviducts.

EXPERIMENTAL METHOD

2,4,6,7-^{3}H-estradiol-17β with a specific activity of 100 Ci/mmole was from the Radiochemical Centre, Amersham. Estradiol-17β (E_2), diethylstilbestrol, synestrol (dihydrostilbestrol), estrone, estriol, and 17α-ethinylestradiol were from Calbiochem, and estradiol 3-benzoate, estradiol-17β-valerate, 2- and 4-Br-estradiols, L-estradiol, 17- and 3-deoxyestradiols, and 3-deoxyestrone were provided by Candidates of Chemical Sciences G. S. Grinenko and K. K. Pivnitskii. 0.01 M Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, 0.25 M sucrose, activated charcoal Norit A, repeatedly washed with water and dried for 24 h at 100°C, as a 0.25% suspension in buffer containing 0.1% gelatin, and 0.5 g POPOP and 5 g PPO in 1 liter toluene as the scintillator, were used. Sexually immature female guinea pigs weighing 150-200 g were decapitated, the tubes were removed on ice, cut into small pieces, and then homogenized in a glass homogenizer in 10 volumes of buffer, after which the homogenate was filtered through a Kapron filter and centrifuged for 1 h at 105,000 g. The residue was used for determination of DNA [3]. Aliquots of cytosol were kept for 1 month at −30°C. The dissociation velocity constant (k_{-1}) was determined by the method of Mester and Robertson [10], using a charcoal suspension and excess of unlabeled hormone. The half-life of the complex was determined by the equation $T_{1/2} = \ln 2/R_{-1}$ for reactions of the second order [2]. To determine the equilibrium association constant (K_{ass}) of E_2 with the isolated R system the principle of saturation analysis was used. K_{ass} and the concentration of combining sites were calculated by Scatchard's method [15]. The range of concentrations of labeled E_2 was 0.05-1.5 pM. The free and bound fractions of steroids were separated by means of the charcoal suspension. To calculate nonspecific binding, parallel with saturation with E_2-^{3}H only, a hundredfold excess of unlabeled E_2 was used [8]. The association velocity constants (k_{+1}) were calculated by the usual formula for reversible

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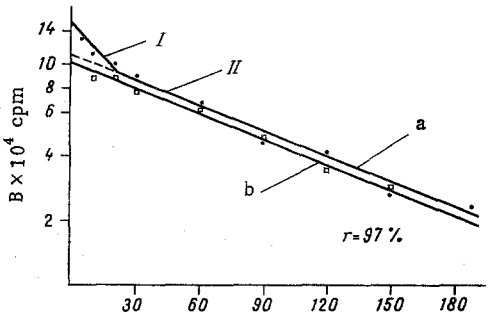

Fig. 1

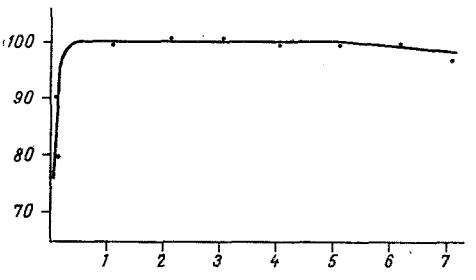

Fig. 2

Fig. 1. Dissociation of estradiol from estradiol-receptor complex at 30°C in the presence of charcoal (a) and an excess of unlabeled estradiol (b). B) Bound ^{3}H-estradiol-17β; r) coefficient of correlation. Ordinate, log B × 10^4 cpm; abscissa, time (in min).

Fig. 2. Kinetics of formation of estradiol-receptor complex. Ordinate, relative percentage (C) of binding of ^{3}H-estradiol receptor system (in %); abscissa, time (in h).

reactions $K_{ass} = k_{+1}/k_{-1}$. The change in the free binding energy of E_2-R was calculated on the basis of the second law of thermodynamics $\Delta G = \Delta H - T\Delta S$ and the consequence from it $\Delta G = -RT\ln K_{ass}$. The relative affinity of the steroids for testing [1] was determined by the use of curves of displacement of E_2 and of two concentrations of the test substance. For statistical analysis of the results and calculation of the physicochemical parameters the Wang 720C programmed calculator was used. A special program was devised for determining the parameters of the Scatchard plot and the number of receptors per cell, with simultaneous statistical analysis based on regression analysis. This program requires no preliminary processing of the results, and the scintillation count, specific activity of the labeled component, counting efficiency, volume of the reaction mixture, and DNA concentration were used as the initial data.* Equilibrium values of K_{ass} of the process of binding of the test substances with the E_2-R system were calculated by Ekins' method [5], using a program drawn up in the writers' laboratory, with K_{ass} of E_2, the dose of labeled E_2 added, and the quantity of bound E_2-^{3}H remaining after displacement by the test substance, and the molar percentage affinity of the test substance as the initial data. To calculate k_{-1} by regression analysis and for the usual statistical analysis, a modification of the corresponding standard programs for the Wang 720C calculator was used.

EXPERIMENTAL RESULTS AND DISCUSSION

Kinetics of Interaction between E_2 and the R System of Cytosol of the Oviducts. Breakdown of the E_2-R complex of the cytosol of the guinea pig oviducts at 30°C is plotted between semilogarithmic coordinates in Fig. 1. Clearly in a typical experiment, during incubation of the E_2-^{3}H-R complex at 30°C with charcoal, curve a consists of two regions, corresponding to fast and slow dissociation (I and II respectively). The rapidly dissociating component of the reaction mixture was a nonspecific complex, which was not further analyzed. The value of k_{-1} for the nonspecific component was analogous to that given for other tissues [2, 10]. Curve b characterizes dissociation of the complex during incubation with an excess of unlabeled E_2 at 30°C. The value of k_{-1} calculated from this curve agrees well with the constant calculated from the "charcoal" curve [$k_{-1} = (0.167 \pm 0.005) \cdot 10^{-3}$ sec^{-1}]. To verify the dependence of the dissociation of the E_2-R complex (formed at 30°C) on temperature, its behavior at 0°C was analyzed. No appreciable dissociation was found under these conditions.

Thermodynamic Characteristics of E_2-R Interaction. As Fig. 2 shows, equilibrium was established in the E_2-R system of the guinea pig oviduct cytosol in 20 min. The E_2-R complex was stable under equilibrium conditions for 5 h at 30°C and only after that did degradation of the complex begin; the mean value of degradation after 7 h was 3%.

To study the E_2-^{3}H-S-R system (in which S is the substance being tested) under conditions approximating to equilibrium, and because of the unknown rate of interaction between S and R, an incubation period of 5 h was chosen as the longest possible on account of the stability of the complex.

*To calculate the molar percentages of affinity with respect to estradiol, the response to the added dose of the test compound was found from the linearized standard curve plotted between logarithmic coordinates [14].

TABLE 1. Physicochemical Characteristics of Estradiol-Receptor Interaction in Cytosol of Guinea Pig Oviducts

Binding	k_{-1} sec^{-1}	$T_{1/2}$ min	K_{ass} M^{-1}	k_{+1} sec$^{-1}\times$M^{-1}	ΔG, kJ / mole	Q, thousands of sites per cell
Specific	$(.160\pm.004)\times10^{-3}$ (n=4)	72	$(.905\pm.221)\times10^{9}$ (n=8)	$.145\times10^{6}$	—52	27,3±3,4 (n=8)
Nonspecific	$(.124\pm0015)\times10^{-2}$	9				

Legend. n) Number of experiments (in each experiment pooled cytosol from 50 animals was used). ΔG and k_{+1} were calculated from mean values of K_{ass} and k_{-1}.

TABLE 2. Characteristics of Affinity of a Series of Steroids for Estradiol–Binding System of Guinea Pig Oviduct (M ± m)

Chemical compound	Molar percentage of affinity (relative to estradiol)	$K_{ass}M^{-1}$
1 Diethylstilbestrol	189,58±10,10	$(0,206\pm0,017)\times10^{10}$
2 Ethinylestradiol-17α	153,91±11,11	$(0,157\pm0,014)\times10^{10}$
3 Synestrol	94,29±8,48	$(0,871\pm0,089)\times10^{9}$
4 Estradiol 17β	100	$(0,905\pm0,221)\times10^{9}$
5 Estradiol-3-benzoate	48,94±3,2	$(0,424\pm0,032)\times10^{9}$
6 Estradiol-17β-valerate	12,07±0,86	$(0,987\pm0,063)\times10^{8}$
7 Estrone	8,42±0,91	$(0,710\pm0,072)\times10^{8}$
8 Estriol	6,53±0,65	$(0,509\pm0,048)\times10^{8}$
9 2-Br-estradiol	2,36±0,10	$(0,173\pm0,006)\times10^{8}$
10 4-Br-estradiol	2,04±0,13	$(0,146\pm0,009)\times10^{8}$
11 L-estradiol	1,41±0,09	$(0,869\pm0,165)\times10^{7}$
12 17-deoxyestradiol	0,62±0,03	$(0,411\pm0,017)\times10^{7}$
13 3-deoxyestradiol	0,41±0,03	$(0,266\pm0,019)\times10^{7}$
14 3-deoxyestrone	0,042±0,003	$(0,122\pm0,023)\times10^{7}$

*Values of M ± m calculated from results of six experiments.

The value of K_{ass} and of indices reflecting changes in the free binding energy and the number of combining sites per cell are given in Table 1. The value of K_{ass} for E_2 with the R-system of the guinea pig oviducts was similar to K_{ass} for E_2 with the R-system of the uterus of the guinea pig, rat, and man [1, 11, 13].

Since the concentration of receptors in the reaction mixture does not reflect their number in the cell, the number of receptors per cell was calculated for the test tissue from its DNA content. This value for the tissues of the oviduct agreed in order of magnitude with data in the literature for the human fallopian tubes [13] and for the rat uterus [11].

The change in the free binding energy of E_2 with the receptors of the oviduct cytosol calculated from the experimental values of K_{ass} indicates that the decisive factor in the formation of the complex is the hydrogen bond, in agreement with data in the literature [9, 16].

Specificity of Receptors of Guinea Pig Oviduct Cytosol. Characteristics of the affinity of 14 compounds, expressed as molar percentages relative to estradiol and as values of K_{ass} for interaction between the test compounds and the receptor system were obtained and are given in Table 2. These compounds, as the most active, were selected during investigations of the affinity of a wide range of substances in the receptor system of the guinea pig uterus.

On the basis of these results certain observations can be made on the principles of interaction of estrogens with the E_2-R system of the guinea pig oviduct.

The activity of formation of the steroid-R complex is mainly attributable to integrity of the hydroxyl groups in positions 3 (phenolic) and 17. Transformation of the hydroxyl groups in these positions modifies steroid-R interaction; the degree of change is evidently determined by the nature of the transformation. For instance, removal of the 17β-OH-group (compound 12) sharply reduces the activity of complex formation, whereas oxidation (compound 7) or esterification with valeric acid (compound 6) reduces the degree of steroid-R interaction much less. Removal of the 3-OH-group (compound 13) reduces steroid-R interaction much more than esterification of that hydroxyl group with benzoic acid, and under these circumstances the possibility of hydrolysis of steroid esters to estradiol must be remembered. Moreover, the immediate environment is not without its effect on the hydroxyl groups in positions 3 (phenolic) and 17: introduction of an ethinyl group in position 17α (compound 2) increases the percentage of affinity of the steroid for the test system, whereas introduction of a hydroxyl group in position 16 (compound 8) reduces it. The marked decrease in the activity of steroid-R interaction after introduction of bromine in positions 2 and 4 (compounds 9 and 10) may perhaps be due to the formation of intramolecular hydrogen bonds between the halogen and the OH group or with steric screening of that group by the bromine atom.

The mirror isomer of estradiol (compound 11) is characterized by low binding ability with the R system.

Nonsteroid compounds showed high affinity for the receptor system studied: in the case of synestrol (dihydrostilbestrol) the affinity was almost equal to that of estradiol, whereas for diethylstilbestrol it was twice as high (compounds 1 and 3).

LITERATURE CITED

1. R. N. Shchedrina, S. V. Sturchak, E. G. Bobrova, et al., Byull. Éksp. Biol. Med., No. 8, 989 (1976).
2. M. Best-Belpomme, J. Frier, and T. Erdios, Eur. J. Biochem., 17, 425 (1970).
3. K. Burton, Biochem. J., 62, 315 (1956).
4. R. A. Cowan, U. H. Kim, and G. C. Mueller, Steroids, 25, 135 (1975).
5. R. P. Ekins et al., in: Radioisotopes in Medicine: In Vitro Studies (ed. by R. L. Hayes et al.), Oak Ridge (1968), p. 59.
6. E. E. Baulieu, Biochem. Pharmacol., 24, 1743 (1975).
7. M. Ginsburg, B. D. Greenstein, N. J. MacLusky, et al., Steroids, 23, 773 (1974).
8. A. J. Hsueh, E. J. Peck, jr., et al., Steroids, 24, 599 (1974).
9. R. J. B. King and W. I. P. Mainwaring, Steroid–Cell Interactions, Univ. Park Press, Baltimore (1974), p. 7.
10. J. Mester and D. M. Robertson, Biochim. Biophys. Acta, 230, 543 (1971).
11. A. C. Notides, Endocrinology, 87, 987 (1970).
12. E. J. Pavlik and P. B. Coulson, J. Steroid Biochem., 7, 369 (1976).
13. D. M. Robertson, B. M. Laudgren, and R. Guerrero, Acta Endocrinol. (Copenhagen), 80, 705 (1975).
14. D. Rodbard, W. Bridson, and P. L. Raybord, J. Lab. Clin. Med., 74, 770 (1969).
15. G. Scatchard, Ann. N. Y. Acad. Sci., 51, 660 (1949).
16. U. Westphal, Steroid–Protein Interaction, Springer-Verlag, Berlin (1971).