CHARACTER OF BINDING OF SOME ESTROGENS WITH ESTROGEN–RECEPTOR SYSTEMS IN THE HYPOTHALAMIC–HYPOPHYSEAL STRUCTURES OF GUINEA PIGS

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The attention of research workers in recent years has been drawn to the study of the steroid-receptor apparatus of the various target organs of the reproductive system. The properties of the estrogen-receptor (ER) systems of the uterus and oviducts of different species of animals have been more or less fully characterized by investigations including some from our own laboratory [1-6].

Data on the characteristics of ER-interaction in the CNS are very few in number and contradictory in nature. Specificity of interaction in these structures has been inadequately studied.

In a previous study [7] the writers analyzed the basic physicochemical parameters of ER-interaction in the adenohypophysis and anterior hypothalamus. The object of the present investigation was to study the specificity of ER-interaction in the cytosol of these same structures.

EXPERIMENTAL METHOD

Reagents. 2,4,6,7-^{3}H-estradiol-17β, with specific activity 115 Ci/mmole, was from Radiochemical Centre, Amersham, England; nonradioactive estrogens were obtained from the same sources as previously [1-4]; Tris-HCl buffer was used in a concentration of 0.1 M, EDTA 1 M, sucrose 0.25 M, pH 7.4; 0.25% activated charcoal (Norit A) was suspended in buffer containing 0.1% gelatin.

To obtain ER-systems of the adenohypophysis and anterior hypothalamus tissues from sexually immature guinea pigs weighing 200-250 g were used. The tissues were isolated according to the generally accepted topographic schemes [8].

Cytosols were obtained by centrifugation of tissue homogenates diluted with buffer – in the ratio of 1:20 for the hypophysis and 1:1 for the hypothalamus, at 105,000g for 60 min.

The specificity of ER-interaction was studied by the scheme described previously [3], using the principle of saturation analysis [9]. The relative affinity of the compounds was calculated relative to the affinity of estradiol, which was taken as unity. Linearized [10] displacement curves were used for the calculations (Fig. 1). The concentrations of the nonradioactive compounds studied were chosen so that the values of their displacing effects lay within the working interval (linearized region) of the standard curve.

For the closest approximation to a state of equilibrium in the estradiol (the hormone studied in this case)–receptor system, incubation at 30°C for 5 h was used. This time was chosen on the basis of data in [7] on the stability of the ER-complex of the tissues studied. No significant degradation of the receptor systems was observed under these conditions.

Computation of the standard curves, calculation of the equilibrium constants ($K_{ass.}$), and statistical analysis of the results were carried out on a "Wang 720C" programmed calculator, using both standard programs and others developed in the writers' laboratory.

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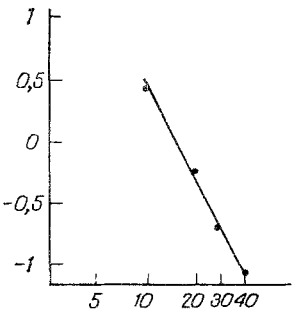

Fig. 1. Typical standard curve of displacement of estradiol from estradiol-receptor complex of cytosol of anterior hypothalamus. Abscissa — log dose (in pg); ordinate — ln relative percentage of binding. Sensitivity of curve 2.5 pg, slope 2.49.

EXPERIMENTAL RESULTS

The estrogen preparations used in the investigation included compounds with modifications to the main functional groups of the steroid molecule (Table 1). The table includes nine compounds with modifications to the estradiol molecule and three synthetic preparations, two of which possess high estrogenic activity (compounds 1 and 2), whereas the third (clomiphene) is an antiestrogen with supposedly central action, widely used in clinical practice,

The results showed that the basic principles of ER-binding established for the uterus and oviducts [3, 4] apply also to hypothalamo–hypophyseal structures. Analysis of the results (Table 1) shows the significant role of the 3rd phenol group and the 17β-hydroxyl groups in ER-interaction in these tissues.

In fact, any changes in the structure of the steroid molecule in accordance with the principles stated above and in neighboring positions, such as oxidation of estradiol to estrone (compound 5), the introduction of a hydroxyl group in the 16α-position (compound 4) or or an ethinyl or ethyl group in the 17α-position (compounds 3 and 8), bromination in positions 2 and 4 (compounds 6 and 7), and reduction of the above-mentioned hydroxyl groups (compounds 9 and 10) — significantly modify the affinity of the estrogens for the R-systems studied in the guinea pigs.

The substituents used affected the degree of change in the affinity of the estrogens for the R-systems differently. For instance, introduction of the 16α-hydroxyl group into the estradiol molecule reduces affinity only half as much as oxidation of the 17β-hydroxyl group (compounds 4 and 5), but introduction of an ethyl group in the 17α-position reduces the affinity of the steroid molecule by half again (compound 8). Complete reduction of the 17th and 3rd hydroxyl groups in the estradiol molecule (compounds 9 and 10) leads to even greater loss of ability of the estrogen to bind with the receptors of the above target organs. Modification of both hydroxyl groups simultaneously was accompanied by the greatest decrease in affinity of the estrogen for the cytosol receptors of hypothalamo–hypophyseal structures (compound 12). By contrast with the substituents mentioned above, an ethinyl group in the 17α-position significantly increases the affinity of the estrogen for these R-systems (compound 3). Synthetic estrogens — diethylstilbestrol and dihydrostilbestrol (compounds 1 and 2) — interact with receptors of the adenohypophysis and anterior hypothalamus more actively than the natural hormone estradiol, whereas the antiestrogen clomiphene has relatively low affinity for these R-systems.

Comparison of these results with data in the literature is difficult because of the almost total absence of such data. The only information in the literature, on interaction between stilbestrol, ethinylestradiol, estrone, estriol, and clomiphene and ER-systems of the CNS, does not contradict the principles established by the present writers for the hypophysis and hypothalamus [11-13]. However, these published data were obtained by nonquantitative methods, so that any closer comparison is ruled out.

Attention must be drawn to significant differences in the affinity of some of the estrogens studied for the R-systems of the adenohypophysis and anterior hypothalamus: Compounds 3, 4, and 6 reacted more actively with the R-molecules of the hypothalamus, whereas compounds 9, 10, and 11 showed greater affinity for the R-systems of the hypophysis, and the synthetic

TABLE 1. Affinity of a Series of Estrogens for ER-Systems of Hypothalamo-Hypophyseal Structures (M ± m)

Compound	Percentage affinity		K_{ass}		P
	hypophysis	hypothalamus	hypophysis	hypothalamus	
1 (diethylstilbestrol)	166±9	133±13	0,209±0,012 $\times 10^{10}$	0,272±0,028 $\times 10^{10}$	Not significant
2 (synestrol)*	163±19	102±7	0,204±0,023 $\times 10^{10}$	0,206±0,014 $\times 10^{10}$	"
3 (17α-ethynylestradiol)	145±9	133±16	0,183±0,012 $\times 10^{10}$	0,271±0,034 $\times 10^{10}$	0,025
4 (estriol)	4,0±0,3	5,0±0,7	0,473±0,032 $\times 10^{8}$	0,982±0,152 $\times 10^{8}$	0,0025
5 (estrone)	2,5±0,28	2,5±0,23	0,289±0,033 $\times 10^{8}$	0,479±0,044 $\times 10^{8}$	Not significant
6 (2Br-estradiol)	2,21±0,03	1,79±0,01	0,259±0,002 $\times 10^{8}$	0,332±0,001 $\times 10^{8}$	0,0005
7 (4Br-estradiol)	1,9±0,3	1,01±0,05	0,218±0,032 $\times 10^{8}$	0,186±0,012 $\times 10^{8}$	Not significant
8 (17α-ethylestradiol)	1,09±0,06	0,76±0,15	0,135±0,012 $\times 10^{8}$	0,140±0,029 $\times 10^{8}$	" "
9 (17-deoxyestradiol)	0,95±0,1	0,40±0,05	0,116±0,012 $\times 10^{8}$	0,788±0,106 $\times 10^{7}$	0,0025
10 (3-deoxyestradiol)	0,49±0,04	0,23±0,09	0,526±0,044 $\times 10^{7}$	0,449±0,050 $\times 10^{7}$	0,025
11 (clomiphene)	0,49±0,06	0,15±0,00	0,486±0,074 $\times 10^{7}$	0,278±0,009 $\times 10^{7}$	0,0125
12 (3-deoxyestrone)	0,018±0,001	0,012±0,002	0,200±0,015 $\times 10^{6}$	0,227±0,036 $\times 10^{6}$	Not significant

*Dihydrostilbestrol.

antiestrogen clomiphene also displayed higher affinity for R-systems of the hypophysis, which suggests the possibility that the action of this compound may be selective,

The results evidently reveal definite differences in the structure of the estrogen-binding site of receptors of the hypophysis and hypothalamus,

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