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SPECIFICITY OF THE ESTRADIOL-BINDING SYSTEM OF THE GUINEA PIG UTERUS

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The degree of affinity of steroids of the estrone series for the estradiol-binding system of the guinea pig uterus was analyzed. The factor determining interaction with the receptor system of the guinea pig uterus was found to be the presence of free hydroxyl groups in positions 3 (phenol) and 17β and their mutual orientation. The results suggest that the biological activity of steroids is determined by the character of their structural interaction with the receptor systems of the uterus.

KEY WORDS: *Estradiol-binding system; steroid-receptor interaction.*

Much attention is now being paid to the mechanism of interaction of hormones with tissue receptors [8-10].

In this investigation the properties of the estradiol-binding system, identified previously [4] in the uterus of guinea pigs, were studied.

EXPERIMENTAL METHOD

The estradiol-binding system consisted of the 105,000g cytosol and the 800g supernatant of the uterus of sexually immature guinea pigs [4]. The same general pattern is observed when both fractions are used [4, 13]. The protein content in the system was determined by Lowry's method [12]. The following substances were used: estradiol-17β-6,7-^{3}H (specific activity 56 Ci/mmole, Radiochemical Centre, Amersham, England) and unlabeled steroids (Fig. 1) — estradiol (No. 1), estrone (No. 5), estriol (No. 7), ethinylestradiol (No. 2; Calbiochem, USA), and L-estradiol, Jenapharm, East Germany; steroids Nos. 9, 11-13, 15-17, 19-43, and 45-48 (all of the D-series) were obtained in the course of the writers' synthetic investigations [1-3, 5, 6] and they had constants which corresponded to data in the literature. The remaining steroids were obtained from the Laboratory of Steroid Hormonal Chemistry, All-Union Pharmaceutical Chemical Research Institute.* Radioactivity was measured on a Tricarb 3320 liquid scintillation spectrometer with a counting efficiency as ^{3}H of 50%.

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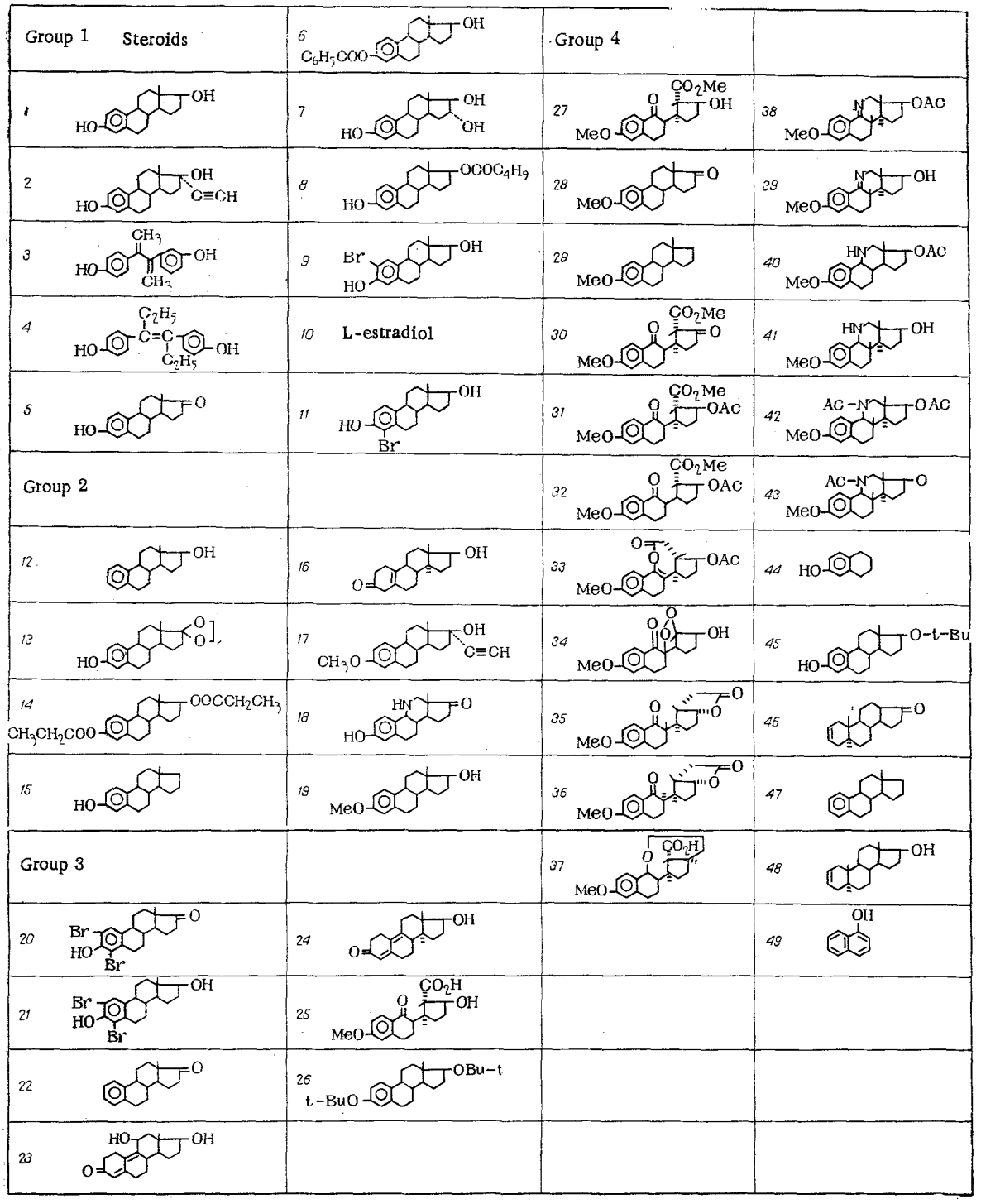

Fig. 1. Structural formulas of steroids tested.

EXPERIMENTAL RESULTS AND DISCUSSION

To determine the binding power of the uterine tissues increasing concentrations of estradiol-17β-6,7-^{3}H (50-600 pg) were used. The results were plotted as Scatchard curves, from which the number of binding sites of the isolated system (of the order of $0.50 \cdot 10^{-12}$ M/mg protein) was determined and the association constant calculated for estradiol relative to the protein of that system.

The affinity of the test steroids was determined by the usual method with the aid of displacement curves (Fig. 2). To estimate the affinity of steroids with an activity close to that of estradiol, curves of displacement of labeled estradiol by these compounds were used. The affinity of relatively inactive steroids was assessed for concentrations of them 50 times greater than the maximal concentration of estradiol-17β.

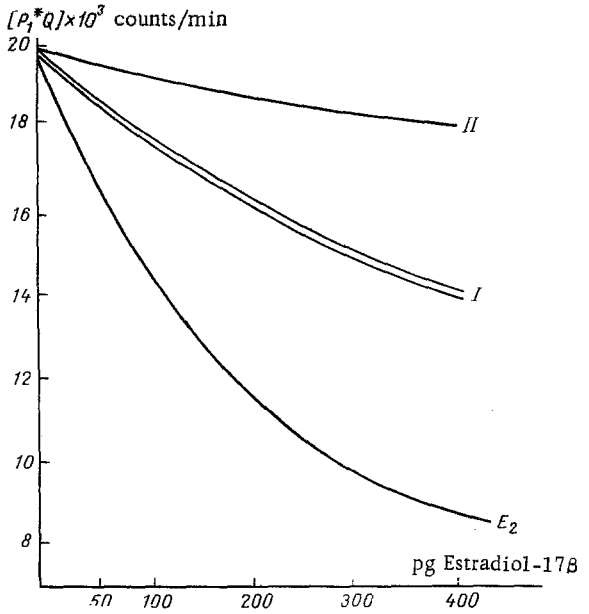

Fig. 2. Standard displacement curve for estradiol-17β and other steroids. E_2) estradiol-17β; I) estrone; II) 3-methyl ester of estradiol.

TABLE 1. Relative Affinity and Association Constants (K_{ass}) of Various Steroids with Estradiol-Binding System of Guinea Pig Uterus

Group No.	Compounds	K_{ass}, M^{-1}/mg protein/liter	log K_{ass}	%
1	Estradiol-17β	$5,6 \cdot 10^8$	8,7	100
	17α-Ethinyl-estradiol	$4,2 \cdot 10^8$	8,6	83,1
	Dihydrostilbestrol	$3,1 \cdot 10^8$	8,5	76,0
	Diethylstil-bestrol	$2,1 \cdot 10^8$	8,3	55,4
	Estrone	$1,2 \cdot 10^8$	8,1	26,0
	Estradiol-3-benzoate	$1,1 \cdot 10^8$	8,0	18,0
	Estriol 17 valerianate	$2,1 \cdot 10^7$	7,3	9,4
	Estradiol	$1,9 \cdot 10^7$	7,3	6,35
	4-Bromestra-diol	$1,7 \cdot 10^7$	7,2	2,0
	L-Estradiol	$1,3 \cdot 10^7$	7,1	1,4
	2-Bromoestra-diol	$11,\cdot 10^7$	7,0	1,9
2		$7,6 \cdot 10^6$—$2,8 \cdot 10^6$	6,9—6,4	1
3		$1,6 \cdot 10^6$—$4,3 \cdot 10^5$	6,2—5,6	~0,1—1
4		$<3,3 \cdot 10^5$	<5,5	<0,1

To assess the specificity of the various compounds, the method used to analyze the results is of great importance. The method of expressing the result for affinity for the receptor as a percentage, the affinity of estradiol being taken as 100% [11], is now generally used. An attempt was made to characterize the degree of affinity of a steroid for the system not only by the percentage of affinity, but also by the association constant, calculated by the method suggested by V. L. Ishkov, on the staff of the Institute of Rheumatism, Academy of Medical Sciences of the USSR.

Two reversible reactions taking place in the system: $P_1^*+Q=P_1^*Q$ and $P_2+Q=P_2Q$ are described in the equilibrium state by five equations: $[P_1^*Q]=k_1[P_1^*][Q]$; $[P_2Q]=k_2[P_2][Q]$; q=

$[Q]+[P_1^*Q]+[P_2Q]$; $P_1^*=[P_1^*]+[P_1^*Q]$; $P_2=[P_2]+[P_2Q]$, by the transformation of which the expression

$$K=\frac{(q-a-[P_1^*Q])}{P_2-q+a+[P_1^*Q]}\cdot\frac{1}{a}$$

can be obtained.

In this equation all the values are known before the experiment or are determined in its course. The meanings of the terms are as follows: q the concentration of binding sites; [Q] the equilibrium concentration of free binding sites; P_1^* the concentration of added labeled hormone; $[P_1^*]$ the equilibrium concentration of free labeled hormone; P_2 the concentration of added unlabeled hormone; $[P_2]$ the equilibrium concentration of the free unlabeled hormone; $[P_1^*Q]$ the equilibrium concentration of bound labeled hormone measured in the experiment; P_2Q the equilibrium concentration of bound unlabeled hormone; k_1 the constant of affinity of the first (labeled) hormone for the particular binding system; k_2 the constant of affinity of the second (unlabeled) hormone for the particular binding system;

$$a=\frac{[P_1^*Q]}{(P_1^*-[P_1^*Q])\cdot K_1}.$$

Percentages of affinity of the hormones and the calculated association constants of the hormones with the estradiol-binding system of the guinea pig uterus are given in Table 1.

The results suggest that the presence of free hydroxyl groups in the positions 3 (phenol) and 17β and their mutual orientation (L-estradiol, No. 10) are decisive for interaction with the receptor system of the guinea pig uterus. These results agree with those obtained for the binding system of the human uterus [7]. Removal or chemical modification of only one of these hydroxyl groups reduces the association constant 100-fold or more, and changing both groups leads to a further decrease in affinity. The chemical nature of the transformation of these hydroxyl groups (removal, methylation, conversion to a keto group, and so on) has relatively little bearing on binding. In other words, it is only the hydroxyl groups in positions 3 and 17 that are responsible for interaction between the steroids and the receptor protein. It is also essential that the phenolic hydroxyl group preserve the same immediate environment (see Nos. 9, 11, and 20 in Fig. 1). This suggests that interaction with the two points of the active center of the receptor is essential both for active binding and for the manifestation of the biological activity of the steroid.

Integrity of the steroid skeleton is not essential for active binding of steroids to the receptor system studied (see Nos. 3, 4, and 25 in Fig. 1), but the polar function in the C ring of the steroid sharply reduces binding (see Nos. 18, 27, and 30-43 in Fig. 1). The possibility of transformation of some steroids (Nos. 6, 8, 14, 17, 19, 22, and 28 in Fig. 1) into more active compounds in the course of the experiments likewise cannot be ruled out.

It follows from these results that the biological activity of steroids is determined by the character of their structural interaction with the receptors and systems of the uterus (as can be demonstrated by the absence of estrogenic activity of the 11-azasteroids which do not bind with the receptor) (Nos. 18 and 38-43 in Fig. 1). It can also be postulated that steroids with a modified oxygen function in position 17 will bind with the active center of the receptor only through the phenolic hydroxyl group. Such steroids will thus prevent other steroids, possibly without estrogenic action, from binding with the receptor.

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CULTIVATION OF MOUSE EMBRYONIC LIVER EPITHELIUM

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Epithelial cells of mouse embryonic liver, cultivated in vitro, communicated with each other by highly permeable intercellular contacts, they synthesized α-fetoprotein, and they possessed the property of contact inhibition of phagocytosis. The cultivation technique is described.

KEY WORDS: *Mouse embryonic liver culture; α-fetoprotein; phagocytosis.*

Liver tissue is a favorite object with which to study cellular differentiation and processes of induction and carcinogenesis. A cell system cultivated in vitro, is the optimal model for the study of these processes. Nevertheless, many investigations of this type are carried out in vivo, because of the difficulty of keeping normal epithelium for a long time in vitro. Investigations have recently been published in which epithelial cells have been grown by different methods [8-10, 12, 14].

A method of cultivating mouse embryonic liver epithelium is described in this paper, some characteristics of the resulting culture are given, and the method of identification of epithelial cells of the hepatic parenchyma within the culture is detailed.

EXPERIMENTAL METHOD

The method is a variant of that developed by Éraizer et al. for a cultivation of human embryonic liver [8]. As the method of identification of the epithelial cells the immunohistochemical determination of α-fetoprotein (α-FP) was used, because the production of this substance is known to be a specific function of the hepatic epithelium [1]. Cultures of primary embryonic fibroblast-like cells and primary cultures of mouse embryonic liver were used. The cultures were grown on Eagle's medium with lactalbumin hydrolysate (1:1) and 10% bovine serum. To the medium for cultivating epithelial cells 10% calf embryonic serum and 0.4% glucose were added. To inhibit growth of fibroblast-like cells the medium on which liver cells were seeded contained prednisolone (Gedeon Richter, Hungary) in a concentration of 75 μg/ml. On the day after seeding the medium was changed for one not containing prednisolone. Noninbred mouse embryos aged 13-14 days were used. Cells were grown on coverslips placed in plastic dishes (Linbro, USA).

The α-FP content in the medium was determined by the standard method of precipitation in agar, using a test system [5]. To determine the location of α-FP in the cells the indirect immunofluorescence method [14] was used. Monospecific rabbit antibodies against mouse α-FP, isolated with the aid of immunosorbents, and anti-rabbit donkey serum labeled with fluorescein isothiocyanate, prepared by the Department of Luminescent Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology, were used. The living cultures were fixed by various methods: the standard method of fixation in a mixture of ethanol with glacial acetic

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