NUCLEAR ESTRADIOL RECEPTORS IN SOME NONTARGET ORGANS OF RATS

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UDC 612.815.1:612.621.31

Some properties of macromolecules, specifically binding estradiol (E_2), obtained from KCl extracts of nuclei of the uterus, kidneys, liver, testes, and prostate of male and female rats were investigated. These macromolecules from the uterus and liver were shown to be extractable in the largest amounts from chromatin by KCl in a concentration of 0.6 M. The ability of nuclear extracts of the uterus, kidneys, and liver to bind E_2 specifically was completely abolished by treatment with pronase, but not with RNase or DNase, thus indicating the protein nature of these macromolecules. Only estrogens and not testosterone, 5α-dihydrotestosterone progesterone, or corticosterone, competed for these E_2-binding sites of the macromolecules from nuclear extracts of all the organs tested.

KEY WORDS: nuclear receptors; estrogens; "nontarget" organs.

It is now considered that the action of steroid hormones and, in particular, of estrogens on target organs is effected through an intracellular receptor apparatus. Binding of estrogen with the cytoplasmic receptor causes the transformation of the receptor into a shape suitable for interaction with acceptor sites

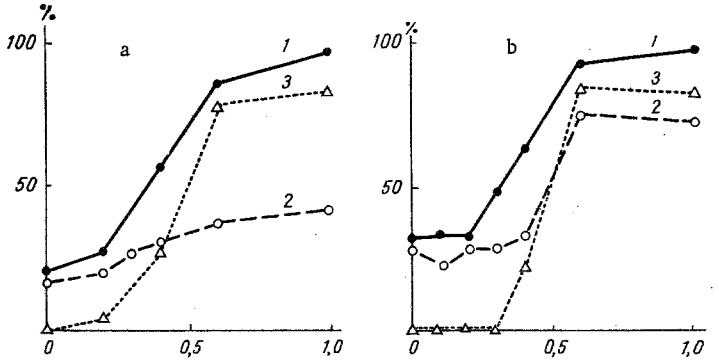

Fig. 1. Extraction of nuclear chromatin macromolecules specifically binding extradiol from the uterus (a) and liver (b) of female rats by different concentrations of KCl in 0.02 M tris-HCl, pH 8.1–1.5 mM EDTA–1.5 mM dithiothreitol buffer. 1) Specifically bound E_2; 2) protein content; 3) DNA content in extracts. Abscissa, KCl concentration (in M); ordinate, specific binding of E_2 in 1.0 M KCl extract taken as 100% for (1), protein and DNA content respectively in original chromatin preparation taken as 100% for (2) and (3).

Laboratory of Endocrinology, Biological Faculty, M. V. Lomonosov University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev,) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 68-70, January, 1976. Original article submitted May 23, 1975.

TABLE 1. Effect of Pronase, RNase, and DNase on Ability of Macromolecules of Nuclear Extracts of Uterus, Kidneys, and Liver of Female Rats to Bind H^3-E_2 Specifically

Organ	Specifically bound H^3-E_2 (in % of control; M ± σ)			
	control	pronase	RNase	DNase†
Uterus	100,0±5,5*	8,0±3,4	94,0±8,2	74,5±14,5
P		<0,001	>0,05	<0,05
Kidneys	100,0±41,9	1,5±51,5	117,5±20,8	106,0±51,0
P		<0,002	>0,1	>0,1
Liver	100,0±12,8	−2,0±15,5	96,5±15,1	83,2±9,9
P		<0,001	>0,1	<0,05

Legend. Values of P given relative to the control.
* Mean values of 6-8 determinations.
†To reduce the proteolytic activity of the preparation of DNase, soy trypsin inhibitor was added to it (2:1).

TABLE 2. Competition between Unlabeled Hormonal Preparations and H^3-E_2 for Binding by Macromolecules of Nuclear Extracts of Some Rat Organs

Hormonal preparations	Ability to compete (in % of ability to compete with E_2; M ± σ)						
	females			males			
	uterus	kidneys	liver	kidneys	liver	testes	prostate
Estradiol	100,0±3,1 (16)	100,0±22,2 (11)	100,0±4,8 (14)	100,0±12,4 (23)	100,0±20,1 (32)	100,0±23,2 (25)	100,0±15,2 (7)
Hexestrol	99,5±3,2 (15)	90,5±19,2 (12)	101,0±4,8 (15)	94,2±14,8 (23)	73,0±14,4 (25)	126,0±21,0 (24)	105,6±11,6 (6)
Estrone	70,0±3,6 (15)	73,5±20,9 (13)	70,8±5,2 (13)	79,9±19,2 (24)	67,0±17,6 (25)	90,1±14,8 (25)	54,4±15,2 (7)
Testosterone	−7,7±5,3 (15)	6,1±28,1 (12)	−6,0±4,0 (15)	−7,9±12,6 (23)	−1,3±32,2 (26)	6,1±15,9 (25)	−2,1±14,9 (6)
5α-Dihydrotestosterone	−0,6±3,5 (16)	−1,4±13,1 (14)	−4,1±3,8 (14)	8,1±12,9 (23)	−0,8±18,7 (26)	0,2±27,5 (21)	4,9±15,2 (7)
Progesterone	−2,8±4,2 (16)	−2,2±24,1 (13)	−5,2±6,3 (14)	−8,6±12,9 (23)	2,7±18,7 (22)	4,9±18,1 (24)	0,4±14,8 (7)
Corticosterone	0,5±3,2 (15)	−5,5±25,2 (13)	−5,3±5,8 (15)	−8,7±12,0 (23)	−0,6±22,8 (34)	1,6±27,4 (21)	0,3±11,2 (7)

Legend. Total number of determinations in control and experimental series shown in parentheses.

of the nuclear chromatin [1, 7]. Estradiol (E_2)-binding proteins similar to the estrogen receptors of the uterus, have also been recently found in the cytoplasm of some "nontarget" organs [2, 3, 5].

It is shown in the investigation described below that nuclear macromolecules discovered previously [3, 5], specifically binding E_2, in a number of nontarget organs also are similar to the nuclear E_2 receptors of the uterus.

EXPERIMENTAL METHOD

Experiments were carried out on adult female and male rats, each of which received 0.2-1 μg E_2 intramuscularly in propylene glycol 2-3 h before sacrifice. The nuclear chromatin of the uterus, testes, prostate, kidneys, and liver of the experimental animals was investigated. Chromatin was obtained and extracted as described previously [4] except that 1.5 mM dithiothreitol was added to the extracting buffer. To determine the degree of specific binding of E_2 and to study the stereospecificity and chemical nature of the E_2-binding macromolecules, 6,7-H^3-estradiol-17β (specific radioactivity 40 Ci/mmole, Radiochemical Centre, Amersham) [2, 3] was used. The protein concentration in the samples was determined by Lowry's method [9] and DNA by Burton's method [6].

EXPERIMENTAL RESULTS AND DISCUSSION

Solubilization of the nuclear E_2 receptors from target organs is usually carried out by means of buffer solutions of high ionic strength [8]. To study the conditions of solubilization of macromolecules from nontarget organs specifically binding E_2 increasing concentrations of KCl (from 0 to 1.0 M) were used, followed by testing of the specific E_2-binding activity of the resulting extracts. As Fig. 1 shows, E_2-binding macromolecules of nuclei from both uterus and liver passed from the chromatin into the buffer solution in all salt concentrations used. In both cases within the KCl concentration range from 0 to 0.2 M the fraction of E_2-binding macromolecules dissolved remained approximately constant (about 30%). Lowering the pH of the buffer from 8.1 to 7.5 considerably reduced the fraction of extractable E_2-binding macromolecules.

With an increase in the salt concentration to 0.6 M, a rapid increase was observed in the fraction of extractable E_2-binding macromolecules; a further increase in the salt concentration (to 1.0 M) had practically no effect. Consequently, nuclear chromatin macromolecules from the uterus and liver specifically binding E_2 thus behaved similarly with respect to solubilization in different concentrations of KCl.

Nuclear E_2 receptors in the uterus are known to be proteins [10]. To discover the chemical nature of the E_2-binding macromolecules of the kidneys and liver, the nuclear extracts were treated with proteases and nucleases, after which their E_2-binding ability was tested. In these experiments (Table 1) only pronase effectively inhibited the specific binding of E_2 by macromolecules of the nuclear extracts from three organs, whereas neither RNase nor DNase had any such effect. Consequently, nuclear macromolecules specifically binding E_2 from the kidneys and liver, like the nuclear E_2 receptors of the uterus, consist at least partly of protein.

The most important characteristic of the estrogen receptors is the high specificity of their affinity for ligands. The results of the experiments to study competition between H^3-E_2 and several unlabeled hormonal preparations for E_2-binding sites on macromolecules of nuclear extracts from various organs are shown in Table 2. Clearly, like the receptors of the uterus, the E_2-binding nuclear proteins of the kidneys and liver of male and female rats (and also of the prostate and testes) can interact only with substances possessing estrogenic activity (estradiol, estrone, hexestrol) and not with testosterone, 5α-dihydrotestosterone, progesterone, or corticosterone. Because of this high stereospecificity and also the high affinity (K_{ac} of the order of 10^9 M^{-1}) and the low capacity for the hormone [3, 5], the E_2-binding nuclear macromolecules of the organs tested can be classed as estrogen receptors.

The cell nuclei of some nontarget organs thus contain receptor molecules which selectively bind E_2 and possess certain common properties with the estrogen receptors of the nuclei of the uterus. Despite the much lower content of E_2-binding macromolecules in the cytoplasm and nuclei of the nontarget organs than in target organs [3, 5], the results may point to some similarity between the mechanism of reception of the hormonal signal in the target and nontarget organs.

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