Like the state of other neuromediator and neuromodulator receptors, the state of atypical receptors (their activation or inhibition) depends on the functional state of the cell and of the organism as a whole. For example, Sakharov [13] has shown that atypical responses to serotonin are recorded on neurons of active snails and are replaced by responses with an increase in membrane permeability in snails in a state of anabiosis.

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EFFECT OF LONG-TERM ESTRADIOL ADMINISTRATION IN DIFFERENT DOSES ON ITS RECEPTORS IN THE RAT UTERUS

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The effect of a single dose of estradiol on the concentration of its receptors in target organs (homospecific regulation) has been studied by many workers [1, 3, 8]. However, it must be pointed out that the stimulating action of the steroid under these circumstances is not the only possible mechanism of regulation by hormones of the level of their receptors in the tissues. A qualitatively different picture of the regulatory effect of estrogens may be obtained by their repeated administration. Under natural conditions target tissues are always subjected to the continuous influence of steroids secreted by the gonads and, in addition, experiments under such conditions provide suitable models of the development of certain pathophysiological states arising during long-term hormone therapy.

The aim of this investigation was to study the effect of long-term administration of different doses of estradiol on the concentration of estrogen receptors in the rat uterus, and to compare it with levels of this hormone in the blood serum and uterine tissue cytosol.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of rats: 1) normal noninbred female rats (age 3-6 months) in the stage of estrus; 2) androgen-sterile females with closed vagina, which were injected on the 2nd-3rd day after birth with testosterone propionate in a dose of 50-100 μg per animal; 3) ovariectomized rats 3-4 weeks after operation. Estradiol benzoate was injected intramuscularly into the rats in the form of an oily solution during the first half of the day for 7-8 days daily in doses of 1 and 10 μg. Control animals were given injections of the pure oily solution. The animals were killed 1 day after the last injection and receptor binding of estradiol in the uterine tissue was determined by the method described in [2,

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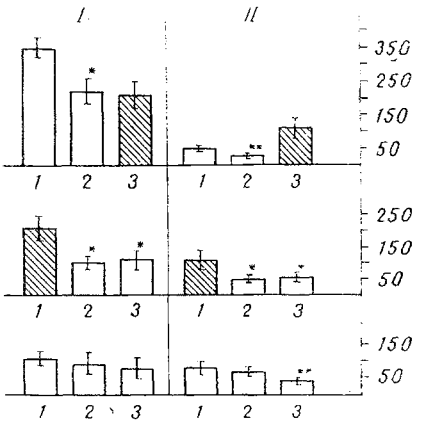

Fig. 1. Receptor binding of [^{3}H]estradiol by cytosol (I) and nucleo-myofibrillary (II) fractions of rat uterine tissue against the background of prolonged (7-8 days) injection of different doses of estradiol (E_2). Above — ovariectomized rats: 1) without injection of E_2, 2) injection of 1 μg E_2, 3) normal rats (stage of estrus) without injection of E_2; middle row — normal rats (stage of estrus): 1) without injection of E_2, 2) injection of 1 μg E_2, 3) injection of 10 μg E_2; bottom row — androgen-sterile female rats: 1) without injection of E_2, 2) injection of 1 μg E_2, 3) injection of 10 μg E_2. Ordinate, binding of [^{3}H]estradiol (in femtomoles/mg protein). *) Difference significant compared with experiment without injection of E_2, at $P < 0.001$ level; **) the same, at $P < 0.01$ level.

7]. For this purpose the rats' uterus was homogenized in the cold. The homogenate was filtered through a nylon filter and part of it was used to prepare low-speed cytosol by centrifugation at 24,000g for 60 min at 0-4°C. The binding reaction was carried out for 18-20 h at 0-4°C with a saturating dose of [6,7-^{3}H]estradiol (specific activity 1.74 TBq/mmole, USSR). To isolate the specific binding component, a 200-fold excess of unlabeled hormone was used. Free and bound forms of hormone were separated by the use of dextran-covered charcoal. The rest of the homogenate was used to study binding of the hormone with the nucleo-myofibrillary fraction of uterine tissue, which was incubated at 37°C for 30 min with 1 picomole of labeled estradiol. The reaction was stopped by the addition of cold buffer. The nuclear fraction was sedimented by centrifugation at 800g (10 min) and the residue was washed with a 50-fold excess of cold buffer. Bound hormone was extracted with absolute ethanol overnight.

The protein concentration in the samples was determined by Lowry's method [5]. The estradiol concentration in blood serum and uterine tissue cytosol from the rats was investigated by a radioimmunologic method using antiserum against estradiol obtained in the laboratory. [6,7-^{3}H]Estradiol of Soviet origin was used as the labeled ligand.

EXPERIMENTAL RESULTS

Injection of estrogens into normal rats for 7-8 days caused a significant decrease in receptor binding of [^{3}H]estradiol by cytosol and nucleo-myofibrillary fractions of uterine tissue almost by half (Fig. 1b). No correlation was found between the degree of decrease of specific binding and the dose of hormone injected (1 and 10 μg). In the ovariectomized rats a significant intracellular redistribution of estrogen receptors took place 3-4 weeks after removal of the ovaries, with an increase in the cytosol (352 ± 42 femtomoles/mg protein) and a sharp decrease in the nuclear (51.0 ± 3.0 femtomoles/mg protein) subfractions compared with their ratio in intact animals in the stage of estrus (211.0 ± 37.5 and 115.5 ± 18.0 femtomoles/mg protein, respectively). Injection of 1 μg estradiol into ovariectomized mice daily for 8 days caused a decrease both in cytosol (220 ± 37 femtomoles/mg protein) and nuclear (26.0 ± 4.1 femtomoles/mg protein) binding of [^{3}H]estradiol by the receptors (Fig. 1a). In androgen-sterile rats with an initially lower content of estrogen receptors than intact animals, inhibition of specific binding of [^{3}H]estradiol during chronic (7-8 days) treatment with exogenous hormone was virtually absent with a dose of 1 μg and weak with a dose of 10 μg (Fig. 1c).

TABLE 1. Uterine Tissue Cytosol and Blood Serum during Prolonged (7-8 days) Injection of Different Doses of Hormone into Rats (M ± m)

Group of animals	Estradiol concentration in cytosol, pg/mg protein	Estradiol concentration in blood serum, picomoles/liter
Normal rats (stage of estrus)		
without injection of E_2	15,3±2,1 (6)	99,1±15,8 (19)
injection of 1 μg E_2	5,9±0,7 (6)	58,7±7,3 (9)
injection of 10 μg E_2	8,6±1,1 (6)	88,1±9,9 (9)
Ovariectomized rats: without injection of E_2	11,4±0,9 (7)	55,1±13,5 (5)
injection of 1 μg E_2	8,8±0,7 (7)	66,0±12,1 (5)
Androgen-sterile rats: without injection of E_2	23,4±2,9 (13)	77,1±14,0 (13)
injection of 1 μg E_2	11,0±1,3 (6)	80,1±6,6 (11)
injection of 10 μg E_2	12,2±1,5 (6)	179,0±77,2 (4)

Legend. Number of rats tested given in parentheses. E_2) Estradiol benzoate.

The inhibitory effect of a long course of estrogen injections on the concentration of estrogen receptors was obtained also in experiments by other workers [6, 9, 10]. It has been shown [10] that this effect is accompanied by inhibition of late responses of the uterus induced by estrogen (incorporation of [^{3}H]thymidine in DNA and of [^{14}C]leucine into protein and oxidation of [^{14}C]glucose into $^{14}CO_2$). The authors cited consider that the prolonged action of estradiol leads to development of metabolic refractoriness of the uterus to further stimulation by the hormone. This reaction of the cytoreceptor apparatus of the target cells evidently develops as a compensatory, protective reaction preventing the entry of excessive quantities of the steroid inside the cells.

In the present experiments the reduction of receptor binding of [^{3}H]estradiol in response to prolonged estrogen administration was observed in all the animals studied (normal, ovariectomized, and androgen-sterile), but the degree of lowering was least in neonatally androgenized rats and the reaction was virtually absent in them when the steroid was given in a dose of 1 μg. This result was evidently due not only to the lower initial receptor concentration, but also to disturbance of the homospecific regulation by the estrogens of the level of their own receptors in the uterus of androgen-sterile rats.

During analysis of the results of these experiments with chronic injection of steroids it was postulated that the excess of endogenous hormone in the tissues, when its action is studied at receptor level, may compete with the isotope and give rise to exaggeration of the degree of exhaustion of receptor binding. To test this hypothesis, besides determining estrogen receptors in the cytosol, we also studied the estradiol concentration.

As Table 1 shows, the estradiol level in the cytosol 24 h after the last injection of the hormone was lower than in rats not given the hormone. The amount of the decrease depended on the dose of hormone. The estradiol concentration in blood sera of these same animals did not differ significantly from the initial level. The results agree with data in [4], in which a special study was made of the time course of steroid hormone concentrations in the blood serum and uterine tissue of mice depending on the dose and mode of administration of the hormones. The animal rapidly utilizes the excess of injected estrogen through activation of enzymes of steroid metabolism in the liver and an increase in their binding with blood transport proteins, so that a relatively constant level of the hormone is maintained and the target cells are protected aginst the harmful action of excessive amounts of steroids.

The experiments described above thus show that exhaustion of estrogen receptors in rat uterine tissue during prolonged exposure to estradiol is not connected with occupancy of the receptors through the raised level of endogenous hormone. This homospecific effect of estrogens can be reduced to self-regulation of the receptor process by a negative feedback mechanism. Relations between hormones and their receptors revealed in target tissues may arise in the development of some forms of endocrinopathies [1] and also during prolonged and intensive treatment with hormonal preparations in the clinical practice.

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