EFFECT OF MALIGNANT TUMORS WITH DIFFERENT CHARACTERISTICS ON STATE OF THE ESTRADIOL-RECEPTOR SYSTEM IN PERIPHERAL TARGET ORGANS

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Variations in the concentration of estrogen receptors in target tissues may be connected with changes in the blood level of these hormones in both physiological and pathological states [6]. However, it is evidently not only the estrogen level which can determine the concentration of receptor sites for these hormones, for it has been shown that other hormones can also affect the concentration of estrogen receptors in target tissues and their translocation in the nucleus [2, 3, 4]. This is also confirmed by the fact that in atrophy of target organs arising as a result of castration, the number of receptor sites in them still remains high and their sensitivity to the corresponding hormones is preserved [9].

However, relations between the concentration of receptors and the action of hormones still remain largely unexplained. At the same time, they are important for the analysis of qualitative changes in reception which may arise and be important in several pathological processes, including in dyshormonal tumors.

The object of this investigation was to study how the steroidogenic function of the ovaries and adrenals changes during growth of primary malignant tumors with different characteristics, and how these changes are reflected in the state of the estradiol receptor system in peripheral target organs and in tissues of the tumor itself.

EXPERIMENTAL METHOD

Experiments were carried out on 24 noninbred rats with normal estrous cycles, weighing 150-160 g, and with a transplanted tumor growing in them. Two different strains of tumor were studied: mammary gland carcinoma (RMK-1) and lymphosarcoma.

The estradiol and progesterone levels were determined in blood serum obtained from the jugular vein after decapitation of the animals 1-3 weeks after transplantation of the tumor, by radioimmunoassay. The estradiol and progesterone levels in tissue homogenates (ovaries, adrenals) and the estradiol level in the nuclear and cytoplasmic fractions of the uterus, mammary glands, and tumor were determined by a modified method [4]. The concentration of estrogen receptors was determined and the binding constants calculated by the method in [7], and the concentration of nuclear receptors was determined by the usual exchange method [1].

To determine the number of receptors, [2,4,6,7-^{3}H]estradiol-17β, with specific activity 104 Ci/mole, and [1,2,6,7-^{3}H]progesterone, with specific activity of 104 Ci/mole (Radiochemical Centre, Amersham, England), were used after purification by column chromatography on Sephadex LH-20. Estradiol-17β and progesterone, obtained from Calbiochem, USA, served as the standard.

Radioactivity was counted with the Mark III scintillation counter (Nuclear Chicago, USA), with counting efficiency of 58% relative to tritium. Association constants were calculated on the Wang-720 C computer, using standard programs for analysis of Scatchard plots, and the results of radioimmunoassay were calculated by standard programs for this technique.

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TABLE 1. Estradiol and Progesterone Concentrations in Tissues of Rats with Malignant Tumors

Experimental conditions	Estradiol		Progesterone	
	blood, mg/ml	ovaries, pg/g tissue	adrenals, pg/g tissue	blood, mg/ml
Control	42	3260	152	17
Lymphosarcoma	19	1368	176	13
RMK-1	52	3600	400	3

TABLE 2. Estradiol Level in Nuclear and Cytoplasmic Fractions of Target Organs and of Tumor in Rats with Malignant Tumors

Experimental conditions	Uterus				Mammary gland				Tumor			
	cytoplasm		nucleus		cytoplasm		nucleus		cytoplasm		nucleus	
	P	E_2	P	E_2	P	E_2	P	E_2	P	E_2	P	E_2
Control	4520	2880	3 251	2 804	1592	1934	310	3310	—	—	—	—
Lymphosarcoma	708	2646	1 653	999	152	3396	NS	3270	88	1804	NS	902
RMK-1	1475	2600	10 581	17 994	261	NS	2313	3404	346	1875	NS	3607

Legend. P) Number of receptors per cell; E_2) number of estradiol molecules per cell; NS) number of receptors on borderline of sensitivity of method.

Estrogen-receptor interaction was assessed on the basis of generally accepted parameters (binding constant, change in the free binding energy).

To compare the concentration of receptors and of estradiol determined in the same fraction, the number of receptors and number of estradiol molecules per cell were calculated by a special formula.

EXPERIMENTAL RESULTS

In all tissues studied highly specific estradiol receptors with high affinity for the hormone and with low capacity were found. This was shown by the values of the binding constants, which varied from $0.1 \cdot 10^{10}$ to $0.7 \cdot 10^{11}$ M^{-1}, and by the change in the free binding energy, which ranged from 52 to 62 kJ/mole.

The investigations showed that in rats with Pliss lymphosarcoma the estradiol level was lowered in the blood and ovaries by more than half compared with the control, and the blood progesterone level was slightly reduced (Table 1). Meanwhile the concentration of cytoplasmic and nuclear receptors was low both in peripheral target organs and in the lymphosarcoma tissue (Table 2).

Growth of the transplanted RMK-1 tumor was accompanied by more complex changes in the steroid-producing function of the ovaries and adrenals, as shown by elevation of the estradiol level, especially in adrenal tissues (more than twofold) and a sharp decrease (almost sixfold) in the progesterone concentration compared with the control (Table 1). Changes in the state of the estradiol-receptor system also differed. The concentration of cytoplasmic receptors fell in the uterus and mammary glands whereas the concentration of nuclear receptors rose sharply, but in tumor tissue the level of cytoplasmic and, in particular, of nuclear receptors was low (Table 2).

The character of changes in the steroidogenic function of the ovaries and adrenals during growth of tumors with different characteristics thus differs, and this is reflected in the state of the estradiol-receptor system of peripheral target organs in the rats of these groups.

Whereas in rats with lymphosarcoma a fall in the estradiol level in the blood and in ovarian tissue coincides with a low concentration of cytoplasmic and nuclear receptors in

the tissues of the uterus and mammary glands and in tissues of the tumor itself, the sharp rise in the content of nuclear receptors in the uterus and mammary glands in rats with RMK-1 carcinoma can evidently be linked with removal of the blocking of estradiol receptor synthesis as a result of a sharp fall in the blood progesterone level and an increase in their synthesis under the influence of the raised estradiol level in the animals of this group. However, it must be noted under these circumstances that the state of the estradiol-receptor system differed, in the uterus and mammary glands on the one hand, and in tissue of the RMK-1 tumor on the other hand.

To discover the role of estrogens in changes in the state of the estradiol-receptor system, the most informative method is to determine the level of the hormone separately in cytoplasmic and nuclear fractions, for this enables the number of receptor sites to be estimated. On this basis, the estradiol level was determined simultaneously with the concentrations of receptors in the cytoplasm and in the nucleus of the uterus, mammary glands, and the tissues of both tumors.

Investigations showed that the ratio of the number of estradiol molecules to the number of receptors per cell in cytoplasm and nucleus could differ in different target organs in the same rats, but could be equal in rats with tumors with different characteristics (Table 2). Meanwhile comparison of the number of receptors in the cytoplasm and nucleus of the lymphosarcoma and RMK-1 tumor revealed a considerable difference between them, even through the level of cytoplasmic and nuclear receptors in both tumors was low. In the case of lymphosarcoma the low level of receptors can be considered to be true, whereas in the case of RMK-1 they may actually be "masked," for the estradiol level in the cytoplasm and nucleus was considerably increased. This suggests that comparison of the number of receptors and the number of estradiol molecules in cytoplasm and nucleus separately would enable the level of occupied receptor sites to be taken into account and, as a result, the effect of the hormones could be characterized more precisely, for example, when estimating the hormone-sensitivity of the tumor.

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