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INTERACTION OF ESTROGEN AND THYROID HORMONES IN THE NUCLEI OF UTERINE TISSUE. **Zorica Žakula[1], S. Đurica[2], S. Radivojša[1], T. Milosavljević[1]** and **Nevena Ribarac-Stepić[1].** [1]*Department of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences,* 11001 Belgrade, Serbia and Montenegro; [2]*Internal Medicine Clinic Zvezdara, University Clinical Centre, Medical Faculty, University of Belgrade*, 11000 Belgrade, Serbia and Montenegro

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Thyroid hormone and estradiol modulate biological processes by binding to nuclear receptor proteins that, through interactions with specific response elements in the regulatory regions of genes, modulate gene transcription. Both the estrogen receptor (ER) and thyroid hormone receptor (TR) are members of the nuclear receptor superfamily (E v a n s , 1988; Z h a n g and L a z a r , 2000). These receptors have a modular protein structure with high homology in the central DNA binding domain (Y e n , 2001). The DNA-binding domain is the most conserved region of the nuclear receptor superfamily responsible for targeting the receptor to highly specific DNA sequences comprising a response element. It is known that ERs bind target response elements with two consensus AGGTCA motifs in a palindromic array with a 3-bp spacer (T r u s s and B e a t o , 1993). However, it has been demonstrated that the same AGGTCA motifs but in other orientations serve as targets for TRs (Y e n and C h i n , 1994). Published studies also indicate that there are two major TR subtypes, TRα- and TRβ- encoded, on separate genes (L a z a r , 1993). With alternative splicing, three functional isoforms, TRα1, TRβ1, and TRβ2, have been identified. The ER exists in two isoforms, α and β, which are products of different genes showing promoter site specificity (K u i p e r *et al.* 1996).

It is known that thyroid hormones have major effects on the female reproductive system (G a r d n e r *et al.* 1978). They are critical for growth, development and differentiation. However, there is insufficient evidence on interactions of these hormones with sex steroids in the cells of reproductive tissues. In order to gain more information about the relationship between thyroid and estrogen hormone actions, we studied receptor binding parameters for both kinds of analyzed hormones, as well as the competition in binding of these hormones to nuclear receptors in the rat uterus.

Adult female rats of the Wistar strain (200-220 g b.w.) were used

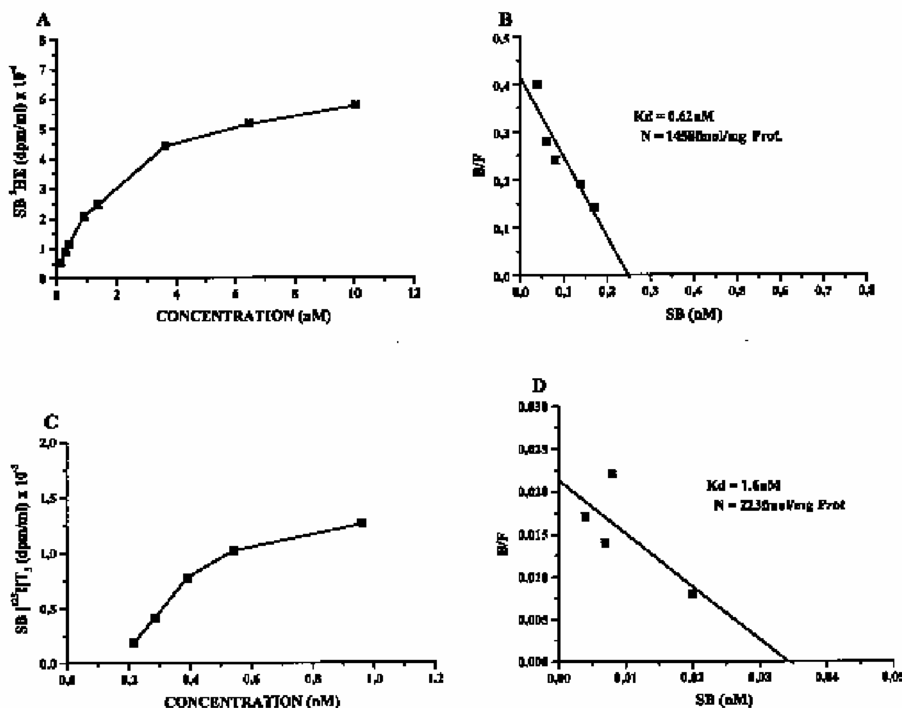

Fig. 1. Saturation curves and Scatchard plots of specific ^{3}HE and $^{125}IT_3$ binding in uterine nuclear extract. Samples were incubated for 2 h at 23°C with the indicated concentration of ^{3}HE (0.125-10 nM) (A and B) or 125IT_3 (0.12-7 nM) (C and D) in the absence or presence of a 1000-fold molar excess of unlabeled Des or T_3. The results represent the mean value of two individual experiments which performed with five females in each of them.

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for the experiments. Animals were ovariectomized seven days before experiments. Nuclear extracts were prepared according to the methods of Evans *et al*. (1983). The animals were killed by decapitation, and the uteri were minced and homogenized in TEMG buffer (50 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 30% glycerol, pH 7.5) using an Ultra-Turrax homogenizer (3x30 sec). The homogenate was filtered through four layers of gauze and centrifuged at 800xg for 15 min at 4°C. The resultant pellet was washed by resuspension and centrifugation twice in TMG buffer (20 mM Tris-HCl, 12 mM monothio-

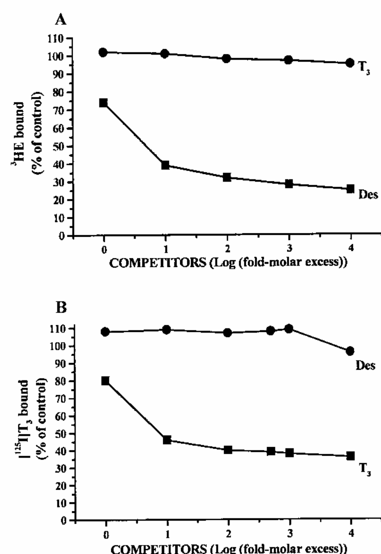

Fig. 2. Competition between ^{3}HE and $^{125}IT_3$ binding in uterine nuclear extract. Samples were incubated for 2 h at 23°C with 6 nM ^{3}HE (A) or 2 nM $^{125}IT_3$ (B) in the absence (control) or presence of increasing concentrations of unlabeled homologous or heterologous competitors. The competitive binding curve is plotted as the percentage of total bound radiolabeled hormones. Each point represents the mean of two separate experiments performed with five animals in each of them.

glycerol, 10% glycerol, pH 7.5) containing 0.2% Triton X-100 and once with TMG buffer alone. The washed nuclear pellet was then resuspended in TEMG buffer containing 0.5 M KCl and mixed at 15-min intervals for 1 h. After centrifugation at 100.000xg for 1 h at 4°C, the obtained supernatant (nuclear extract) was used in the binding studies.

Equilibrium binding studies were done by incubating aliquots of nuclear extracts with increasing concentrations of [2,4,6,7-^{3}H(N)]-estradiol (SA 71 Ci/mmol, Perkin Elmer Life Sciences Inc., Boston, MA) ranging from 0.125 to 10 nM, or ^{125}I-triiodothyronine ($^{125}IT_3$) (SA 67 µCi/µg, Radioisotope Laboratory, Vinča Institute of Nuclear Sciences) ranging from 0.12 to 7 nM in the absence (total binding) or in the presence (non-specific binding) of a 1000-fold excess of unlabeled hormones, diethylstilbestrol (Des) or triiodothyronine (T_3). After incubation, bound and free hormones were separated using the dextran-charcoal absorption binding technique. Receptor binding parameters such as the dissociation constant (Kd) and number of binding sites (N) for both kinds of hormones were determined by Scatchard plot analyses (Scatchard, 1949) using a computer program. Competition assays were done by incubating aliquots of nuclear extracts with a single concentration of labeled hormones and varying the concentration of unlabeled competitors ($1x10^{-9}$ - $1x10^{-4}$ M) for 2 h at 23°C. Protein concentration was measured by the method of Lowry *et al.* (1951).

The obtained results provide evidence for the presence of specific receptors for both analyzed hormones. Binding properties of rat uterus estrogen and thyroid hormone receptors are shown by representative saturation curves and Scatchard plots in Fig. 1. The data indicate the presence of a single class (linear Scatchard plot) of saturable and high-affinity binding sites for both kinds of hormones. The Kd values (Figs.1B and 1D) are in the range observed for ^{3}HE and $^{125}IT_3$ binding in other mammalian tissues (Evans *et al.* 1983). The results of our study also indicate a limited capacity of those binding sites. The T_3 receptor concentration in rat uterus nuclei was much lower in comparison with the concentration of estrogen receptors in the same tissue. This is in agreement with previously published data (Evans *et al.* 1983; Mukku *et al.* 1983) that indicate a small number of T_3 receptors in rat uterus nuclei. Moreover, there are more high-affinity binding sites for T_3, which are responsible for the expected biological effects, in the liver and kidney, than in the uterus (Evans *et al.* 1983).

Since these studies indicate the presence of saturable and high-affinity binding sites in uterine nuclear extracts, binding specificity of these receptors was analyzed using the competitive equilibrium binding method. The results of competition studies are shown in Fig. 2.

Competition experiments revealed that ^{3}HE binding in the nuclear extracts was markedly reduced by the presence of a 1000-fold molar excess of unlabeled Des, but was not reduced by the presence of an equivalent molar excess of unlabeled T_3 (Fig. 2 A). Similar reduction of $^{125}IT_3$ binding was observed in the presence of a 1000-fold molar excess of unlabeled T_3, and there was no competition by Des for $^{125}IT_3$ binding (Fig. 2 B).

Taken together, the data presented herein confirm the presence of specific, high-affinity binding sites for the analyzed hormones in rat uterus, as well as no competition between these two hormones at the receptor-binding level. The mechanisms responsible for the interaction between estadiol and thyroid hormones are not yet known.

In vitro and *in vivo* data indicate that manipulations of estrogen and thyroid hormone levels can alter each other's functions. One possible mechanism for interaction may be that thyroid and estrogen receptors, having structural similarities, bind to an identical half-site, AGGTCA, of their cognate hormone response elements and thereby can compete with each other at this level (Vasudevan *et al.* 2002). It follows that thyroid hormones and estrogen can interact through their own receptors in several different modes, depending on TR and ER isoforms, hormone response elements, and the gene promoter and cell type.

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