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INVESTIGATION OF UTERINE PROGESTERONE RECEPTORS IN RATS DURING ALTERED THYROID ACTIVITY

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ABSTRACT: Our earlier studies demonstrated that thyroid hormone can compensate for a progesterone deficiency during the experimental delay of implantation in rats. The current study investigates whether thyroxin may exert this effect by influencing uterine progesterone receptor levels or receptor binding affinity in rats. Euthyroid, hyperthyroid, and hypothyroid rats were ovariectomized and treated with estradiol. Uterine cytosol was prepared and incubated with ^{3}H-progesterone, an excess of cortisol ± a 100 fold excess of R5020 for 18-24 hours at 4°C. Calculations were made for progesterone receptor picomoles/ng protein and for kinetics of dissociation. Thyroid status did not influence either of these parameters. In our studies, the mechanism by means of which thyroid hormone acts upon uterine physiology does not seem to occur via alteration of the urterine progesterone receptor levels or binding affinity.

INTRODUCTION

Many investigators have reported that thyroid hormone influences the reproductive system and pregnancy in a variety of animal species, including human beings. A review of some of this literature appears in other publications (Archer and Holland, 1981; Evans, *et al.*, 1983; and Leathem, 1972). Despite numerous investigations into this phenomenon, the mechanism by means of which thyroid hormone exerts its effect upon reproduction remains unresolved.

In our laboratory, earlier studies of this phenomenon utilized the technique of experimentally delayed implantation of blastocysts, which allows the investigator to control some of the variables in studies of reproduction (Nutting and Meyer, 1963). Using this technique, it was demonstrated that, in rats receiving daily injections of 48 μg L-thyroxine, the hyperthyroid condition was able to significantly counteract the detrimental effects of progesterone deficiency upon the number of surviving blastocysts on Day 8 of pregnancy or the number of implanting blastocysts on Day 13 of pregnancy. Surgical thyroidectomy had the opposite effect (Holland *et al.*, 1967, 1970). The thyroid effect was shown to be exerted during the progesterone-dependent delay period, rather than during the estrogen-dependent implantation process (Holland *et al.*, 1970). Also, our studies have demonstrated that thyroid hormone can exert effects by acting directly upon the blastocyst (Archer and Holland, 1981) and can act by influencing uterine physiology (Holland *et al.*, 1968).

The goal of the current research in our laboratory is to determine the mechanisms by which thyroid hormone exerts its effects upon female reproductive physiology. The present investigation examines whether the ability of thyroid hormone to compensate for progesterone deficiency during blastocyst maintenance is mediated by an effect upon the uterine progesterone receptor. Several studies in other laboratories have indicated that the uterus may be a target organ for thyroid hormone. Most significantly, thyroid hormone nuclear receptors have been identified in the rat uterus (Evans *et al.*, 1983).

MATERIALS AND METHODS

Sprague-Dawley-derived female albino rats (Harlan-Sprague-Dawley, Indianapolis, IN) between 60 and 120 days old, were maintained on Wayne Laboratory Chow and tap water ad libitum in an animal room at 25°C with a daily illumination schedule of 14 hours of light and ten hours of darkness. Surgical thyroidectomies occurred at least thirty days prior to experimental use of the rats. Hyperthyroidism was induced by daily injection of 48 μg L-thyroxine beginning at least ten days prior to the experiment.

Euthyroid, hypothyroid, and hyperthyroid rats were ovariectomized on Day 1, given subcutaneous injections of 5 μg 17 β-estradiol/200 grams body weight on Days 4 and 5, and were sacrificed by decapitation 24 hours after the last injection.

CYTOSOL PREPARATION

The prodcedure for uterine cytosol preparation was identical to the method of Walter and Clark (1977). This included homogenizing the uteri in 30% TG buffer (10 mM Tris, 30% v/v/ glycerol, pH 7.4) in a cold room using three second bursts of a Polytron homogenizer (Brinkman Instruments) with one minute of cooling between bursts. The final Homogenate was placed in centrifuge tubes and certrifuged at 800 g (2500 rpm) in a Sorval RC2-B centrifuge at 0-4°C for 20 minutes. The supernatant was removed and centrifuged again at 2500 g (26,000 rpm) in a Beckman Model L5-65 ultracentrifuge for 30 min at 0-5°C. Lung, spleen, and diaphragm cytosols were prepared in a similar manner.

SATURATION ANALYSIS AND ONE POINT ASSAY

500 μl aliquots of cytosol preparations were incubated with ^{3}H-progesterone for 18-24 hours at 0-4°C. In the saturation analyses, ^{3}H-progesterone concentrations ranged from either 0.5 mM-30.0 mM or 1.0 mM-20.0 mM. For each radioligand concentration, two total binding tubes and two nonspecific binding tubes were prepared. Nonspecific binding tubes were prepared by adding a 500-fold excess of cortisol plus a 100-fold excess of R5020. R5020 (17, 21-dimethyl-19-nor-4, 9-prenadiene-3, 20 dione) is a synthetic progestin that has a high affinity for the progesterone receptor. For the one point assays, tubes containing 20 mM ^{3}H-progesterone, a 500-fold excess of cortisol ± a 100-fold excess of R5020 were prepared as above.

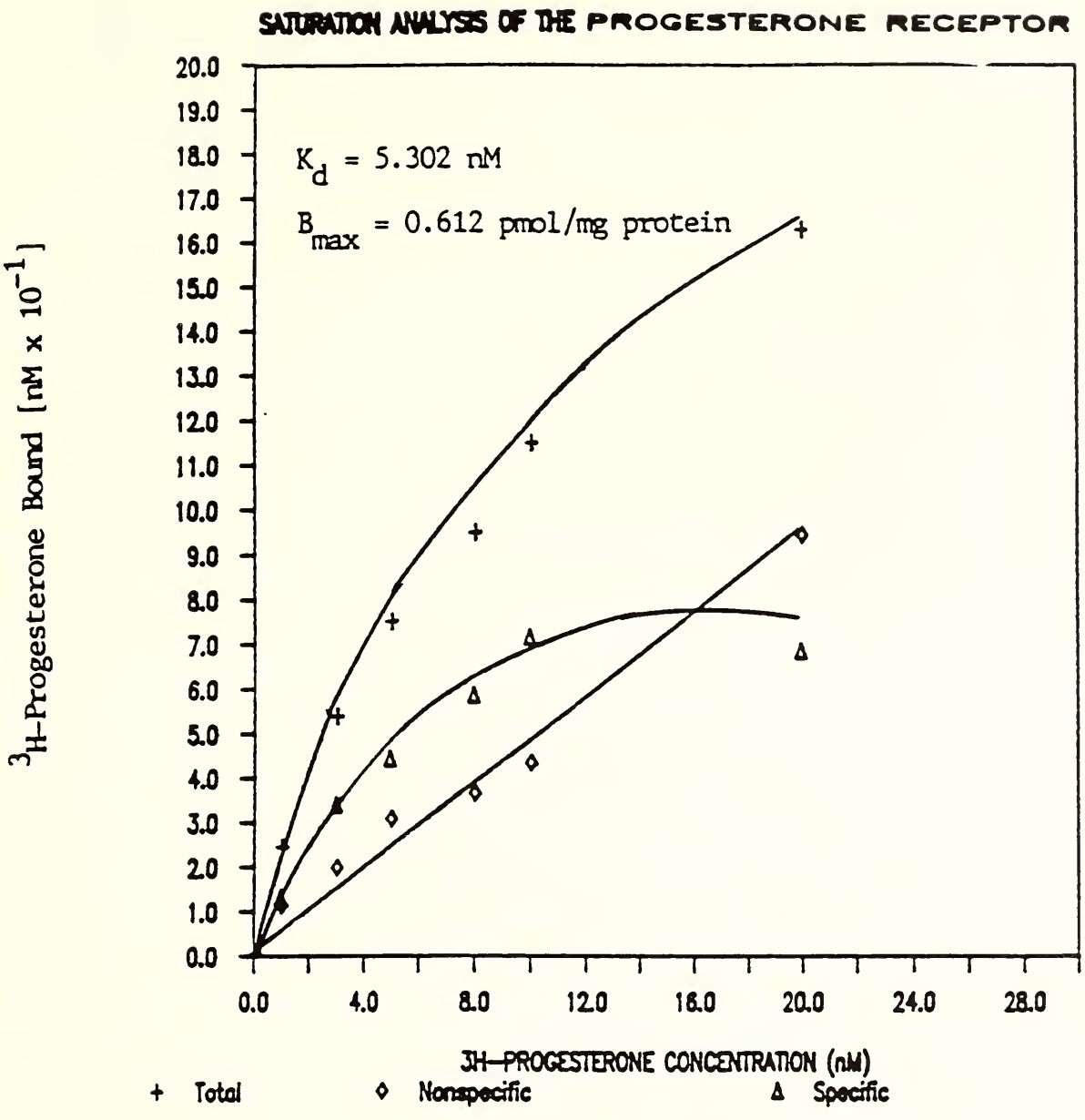
DETERMINATION OF SPECIFICALLY BOND ^{3}H-PROGESTERONE

For the saturation analyses and one point assays, incubations were terminated by brief exposure (30 sec) to 200 μl charcoal suspension (1.0 g charcoal + 0.05 g dextran/ 100 ml 100% TG buffer) and shaking. Tubes were immediately centrifuged in a Sorvall RC2-B certrifuge at 4500 rpm for 6 min at 4°C. 100 μl of the supernatant was pipetted into 5 ml of Aquasol and counted in a Beckman LS 900 Scintillation counter.

EBDA AND LIGAND ANALYSIS

In the saturation analysis experiments, cpms were converted to dpms using the Equilibrium Binding Data Analysis (EBDA) computer program (which can determine the specific activity of radio-labeled steroids). Distintegrations per minute were entered into the EBDA program and estimates of K_d (kinetics of dissociation) and B_{max} (binding affinity) values were obtained. The K_d serves as a measure of the affinity of the pro-

FIGURE 1.



gesterone receptor for progesterone. B_{max} refers to the total number of specific binding sites in the system. The EBDA program was also used to create data files that could be utilized by the LIGAND program. LIGAND is a computer program utilizing non-linear regression analysis (developed by Munson and Robard, 1980) which was employed to obtain reliable estimates of cytosolic progesterone receptor levels and hormone dissociation values (K_d). The K_d and B_{max} estimates and saturation analysis data files (prepared by EBDA) were entered into the LIGAND program. The LIGAND program produced a scatchard plot for each saturation analysis experiment. Protein concentrations were determined by the Lowery protein assay procedure as modified by Peterson (1979).

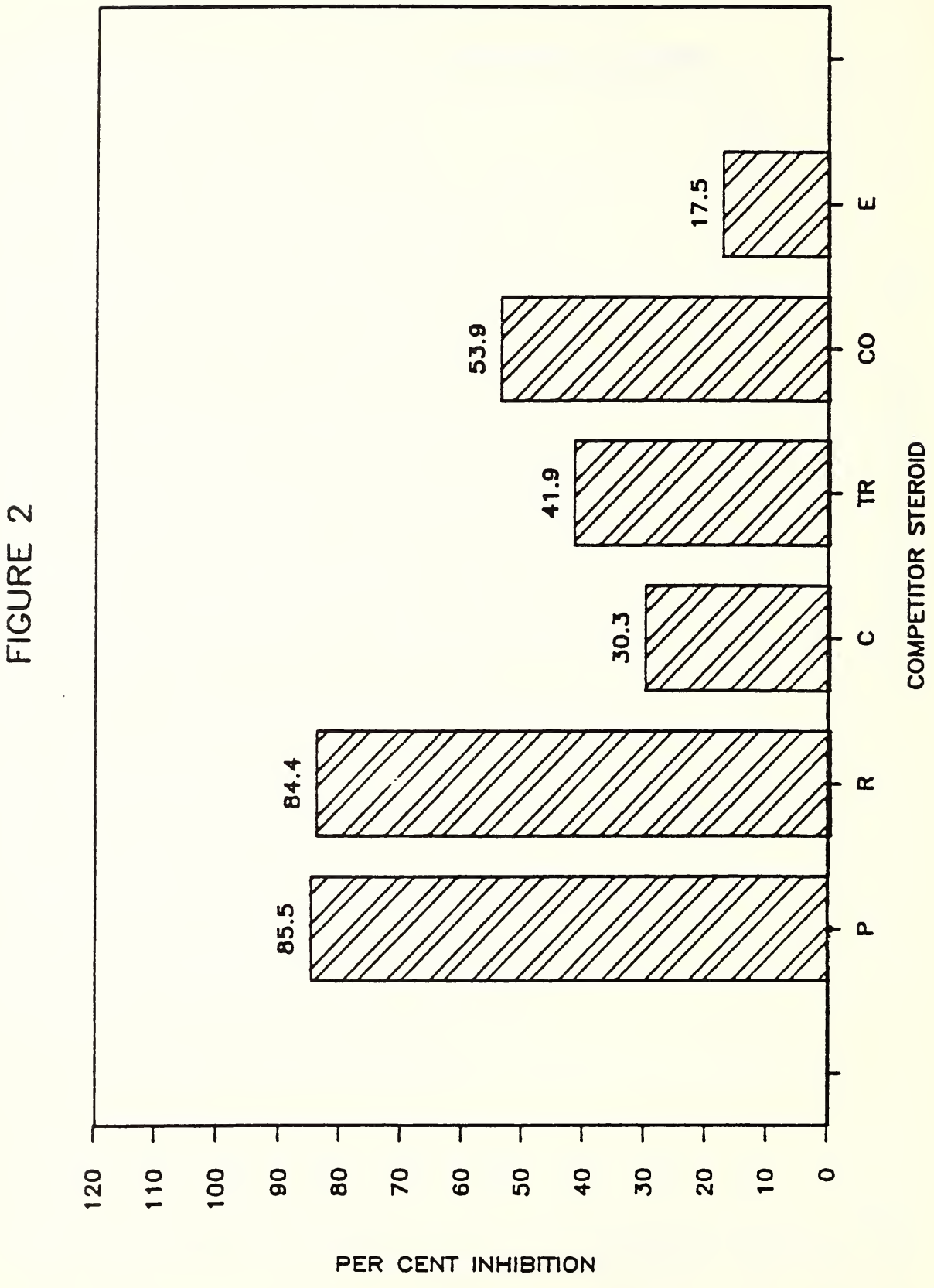


Figure 2. Steroid specificity of the progesterone receptor. Uteri from ovariectomized estrogen-primed rats were homogenized in 30% TG buffer (50 mg tissue/ml buffer), followed by centrifugation and isolation of the cytosol. 500 μl aliquots of the cytosol were incubated with 5 nm ^{3}H-progesterone ± a 100-fold excess of progesterone (P), R5020 (R), cortisol (C), triamcinolone acetonide (TR), corticosterone (CO) or 17β-estradiol (E) for 18-24 hours at 4°C. Two tubes were prepared for each determination of competitor inhibition. Free radioligand was separated by charcoal adsorption, followed by centrifugation and expressed as % inhibition of the total radioligand binding in the uterine cytosol.

Table 1

Thyroid Group	Progesterone Receptor pmoles/mg protein	Kinetics of Dissociation mM
Hypothyroid	0.731 ± 0.016	2.972 ± 0.314
Euthyroid	0.625 ± 0.107	3.560 ± 0.258
Hyperthyroid	0.555 ± 0.141	3.200 ± 0.359

RESULTS AND DISCUSSION

Figure 1, shows a saturation analysis of the progesterone receptor in the uterine cytosol obtained from estrogen-primed, ovariectomized rats. The data from this experiment were subjected to EBDA analysis and the results plotted. These data indicate that saturation of the progesterone receptor was accomplished. The specificity of the radioligand binding in the crude uterine cytosol was examined by allowing 100-fold excess of various steroids (R5020, cortisol, triamcinolone acetate, corticosterone or 17 β-estradiol) to compete with 5 mM of ^{3}H-progesterone for binding sites in uterine cytosol.

The data from steroid specificity of binding studies are presented in Figure 2. Only progesterone and R5020 showed a high percent of inhibition of binding of ^{3}H-progesterone to the uterine cytosol preparation. Very similar results were obtained in other experiments using 20 mM ^{3}H-progesterone. Also, in other experiments, testosterone failed to show any competition for binding sites in the uterine cytosol.

Tissue specificity of ^{3}H-progesterone binding was investigated by incubating 20 mM of radioligand with a 500-fold excess of cortisol ± a 100-fold excess of R5020 with crude cytosol preparations of lung, diaphragm, spleen and uterine tissues. Specific binding was not detected in the cytosols of lung, spleen or diaphragm, while there was a considerable amount of specific binding in uterine cytosol.

Saturation analyses were performed on the uterine cytosol of ovariectomized, estrogen-primed euthyroid, hypothyroid and hyperthyroid rats. Scatchard plots were obtained by EBDA and LIGAND analyses and K_d and B_{max} values were determined. Table 1 shows representative data from these studies which were repeated several times. Data for each thyroid group in Table 1 represent the mean ± standard error of the mean B_{max}/mg protein value or the mean ± s.e.m. K_d value for the assay to two cytosolic preparations. Analysis of variance did not detect a statistically significant difference between the progesterone receptor levels or the kinetics of dissociation in any of the cytosolic preparations for any of the experiments conducted.

In these preliminary experiments, the thyroid status of the rats did not cause a significant change in the amount of uterine progesterone receptor or the binding affinity of the receptor. The ability of thyroid hormone to compensate for a deficiency of progesterone during the maintenance of blastocysts apparently occurs by means of another mechanism. Other possibilities are currently being investigated in our laboratory.

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