Interaction between 16α,17α-Cyclopropane Progesterone and Progesterone Receptors in Rat Uterus

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Binding of ^{3}H-16α,17α-cyclopropanoprogesterone (CPG) and ^{3}H-progesterone (PG) to progesterone receptors in soluble fraction of rat uterus is studied. It is shown that CPG and PG specifically bind to the protein with similar affinity and binding capacity. Unlabeled PG competitively inhibits the binding of CPG, and unlabeled CPG competitively inhibits the binding of PF with the same efficiency. Dissociation of CPG- and PG-receptor complexes is characterized by the same dissociation constant.

Key Words: *progesterone; progesterone receptor; progesterone analog; steroid-receptor interaction*

16α,17α-Cyclopropanoprogesterone (CPG) can be classified as a partial selective agonist, since this substance is superior to progesterone (PG) in the endometrium proliferation test and inferior to PG in the pregnancy maintenance test [1]. To evaluate the mechanism of this phenomenon we synthesized tritium-labeled CPG and compared the interaction of CPG and PG with soluble protein fraction from rat uterus. Our findings argue against the assumption that biological effects of CPG are mediated through its binding to a protein different from classic progesterone receptor.

MATERIALS AND METHODS

Unlabeled PG (Sigma), radiolabeled 1,2,6,7-^{3}H-PG (specific activity 84 Ci/mmol, Amersham), 1,2-^{3}H-CPG synthesized by homogenous catalytic tritiation of 1,2-double bond (specific activity 47 Ci/mmol) and its nontritiated analog [2] were used in the study. Uteri were obtained from intact, estradiol-treated (intramuscular injections of 1 μg in 400 μl estradiol in propylene glycol), and pregnant (gestation days 13-14 or 18-20) rats (180-200 g) from mixed population and stored at -20°C. Uteri were homogenized in 10 mM Tris-HCl buffer (pH 7.5) containing 30% glycerin, 10 mM KCl, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulphonyl fluoride [9]; tissue:buffer ratio varied from 1:2.5 to 1:8 (m/v) depending on the expected PG concentration. This and all subsequent procedures were carried out at 0-4°C. The homogenate was centrifuged at 50,000*g* for 1 h. The supernatant of tissue homogenate from pregnant rats was incubated with dextran-coated charcoal (1 and 0.2% final concentrations) for 30 min followed by centrifugation to remove endogenous gestagens [11]. The supernatant (100-μl aliquots with a protein concentrations of 4-12 mg/ml) was incubated with ^{3}H-ligand in presence for 5 h varied concentrations of unlabeled steroids and 3 mM cortisol for 5 h, after that charcoal was added [3]. Relative competitive activity of test ligands was assessed as described

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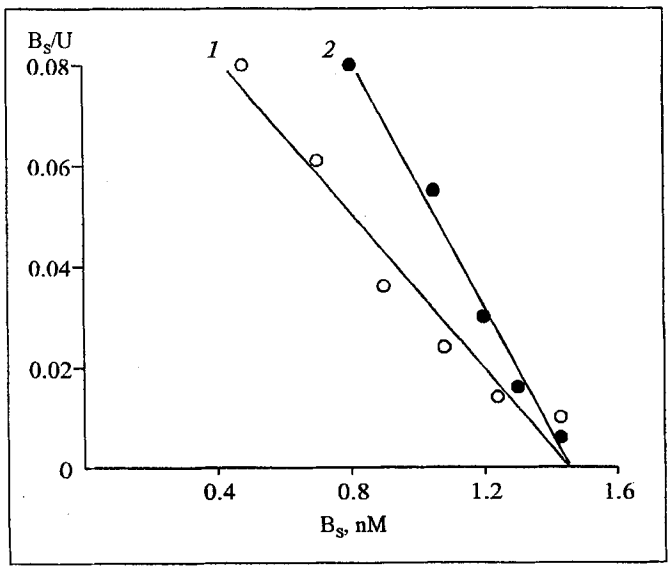

Fig. 1. Scatchard plot of ^{3}H-progesterone (*1*) and ^{3}H-16α,17α-cyclopropanoprogesterone (*2*) with a protein from soluble uterine fraction of estrogenized rat. Final protein concentration was 4.1 mg/ml. Here and in Fig. 2: B_s and U are specifically bound and free ligands.

elsewhere [8]. The steady-state binding constant was measured in Scatchard plots [10]. The steady-state dissociation constant and type of inhibition were assessed from double reciprocal plot [7]. The inhibition constant was calculated from a "slope versus inhibitor concentration" plot [6]. Kinetics of ligand-protein dissociation was analyzed as described previously. Protein concentration was measured by the intensity of Coomassie staining [5]. Steroids were analyzed by thin-layer chromatography in a chloroform:ether 17:3 system [3]. Dilution and incubation of steroids were performed in quartz tubes coated with 3% albumin [3].

RESULTS

Both ^{3}H-CPG and ^{3}H-PG specifically bind to a soluble protein from rat uterus. Scatchard analysis reveals single type of binding sites for both ligands (Fig. 1). Competitive crossover analysis CPG+^{3}H-PG (Fig. 2, *a*) and PG+^{3}H-CPG (Fig. 2, *b*) demonstrated a competitive mutual inhibition of CPG and PG binding and, consequently, both ligands interact with the same binding sites. Dissociation of ^{3}H-CPG-protein and ^{3}H-PG-protein complexes is described by biphasic kinetic curves (Fig. 3). Constants of dissociation rate (k^{-1}) for each phase are similar for both ligands, but the proportions between fast and slow dissociating CPG-protein and PG-protein complexes differ considerably and constitute 11 and 1.2, respectively. Kinetic parameters for CPG-protein and PG-protein interaction are given in Table 1. These parameters are similar for intact, estrogenized, and pregnant rats. The ^{3}H-ligands did not undergo metabolic transformation during incubation.

The use of ^{3}H-CPG allowed us to answer the question on the presence of a tentative specific receptor for CPG different from classic PG receptor in the soluble protein fraction from rat uterus: PG receptor was the only protein that binds specifically both CPG and PG. The use of quartz tubes coated with albumin (reduction of hydrophobicity) almost completely prevented nonspecific adsorption of steroids and markedly elevated measured relative com-

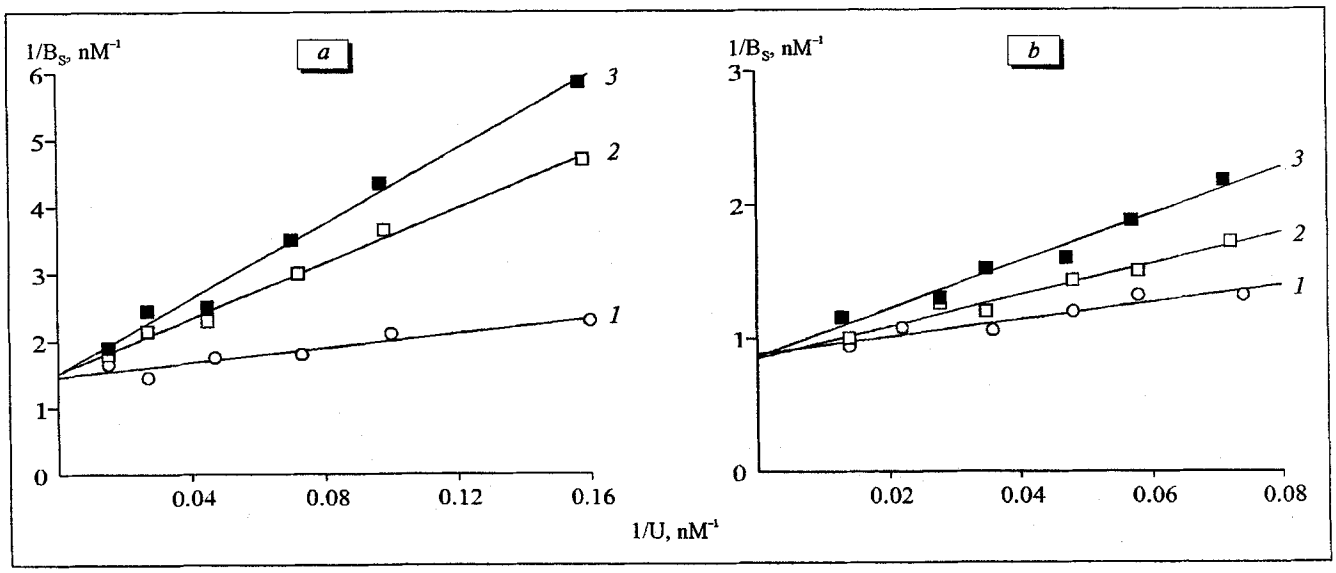

Fig. 2. Inhibition of ^{3}H-progesterone binding to soluble uterine protein of pregnant and estrogenized rats in the presence of unlabeled 16α,17α-cyclopropanoprogesterone (CPG, *a*) and ^{3}H-CPG binding in the presence of unlabeled progesterone (*b*). In the absence of competitor (*1*) and in the presence of 8 (*2*) and 16 nM (*3*) competitor. Final protein concentration 5.1 (*a*) and 2.7 (*b*) mg/ml.

TABLE 1. Kinetic Parameters of Interaction of PG and CPG with Protein from Soluble Fraction of Rat Uterus (*M±m*)

Labeled ligand	Relative competitive activity*	K_i (CPG), nM	K_i (PG), nM	K_d, nM	k_{-1}', $sec^{-1} \times 10^5$
^{3}H-PG	1.02±0.25 (4)	3.5 (2)	—	9.7±2.1 (5)	1.8 (2)
^{3}H-CPG	0.99±0.07 (3)	—	12.2 (2)	15.1±4.1 (5)	3.2 (2)

Note. Activity of progesterone is taken as 1. Number of measurements is shown in parentheses.

petitive activity of CPG in comparison with previous data [4]. Our experiments demonstrated similar affinity of CPG and PG for PG receptor. Hence, the additional cyclic α-substitute in the D ring of PG did not contribute to the total energy of steroid-receptor interaction. On the other hand, analysis of dissociation kinetics of ^{3}H-ligand-protein complexes (Fig. 3) suggests that CPG and PG induce different conformation changes in receptors. These differences between CPG and PG can be related to the above-mentioned peculiarities of their biological effects.

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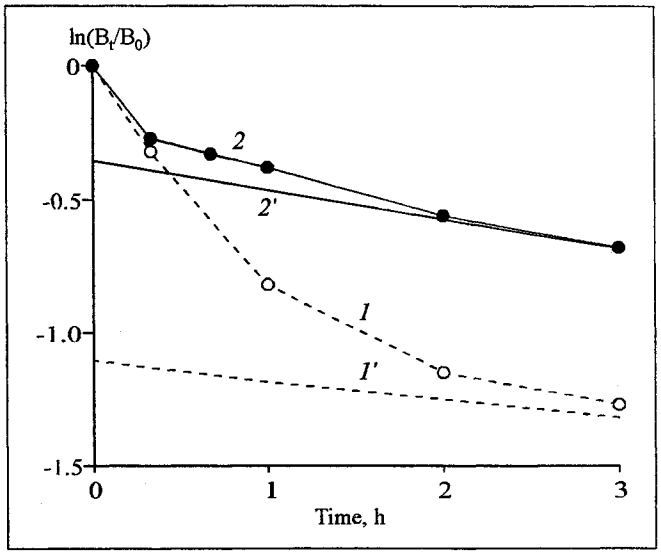

Fig. 3. Dissociation of ^{3}H-progesterone (*1*) and ^{3}H-16α,17α-cyclopropanoprogesterone (*2*) from their complexes with protein from soluble uterine fraction of intact rats. Initial (B_0) and current (B_t) concentrations of specifically bound ligand. Curves *1'* and *2'* are constructed from curves *1* and *2* by subtracting their rapid components. Final protein concentration is 2.7 mg/ml.

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