PHARMACOLOGY AND TOXICOLOGY

Bioactive 5-Reduced 16α,17α-Cyclohexanoprogesterone Derivatives Weakly Interact with Proteins of the Soluble Uterine Fraction

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We studied competitive activities of 16α,17α-cyclohexano-5α- and 5β-dihydroprogesterone in replacing ^{3}H-progesterone and ^{3}H-16α,17α-cyclohexano-6α-methylprogesterone from protein complexes. Direct binding of ^{3}H-5-reduced derivatives with proteins of soluble fractions from rat and rabbit uteri was also assayed. C_d values for 5-reduced derivatives were in the micro- or submicromolar range. The data suggest that biological effects of these analogues are not mediated via soluble uterine receptors.

Key Words: *progestins; steroids; analogues; receptors; binding*

Pregna-D'-pentaranes are progesterone derivatives containing additional 16α,17α-carbocycles and possessing high progestogen activity (in some cases, selective) [1,4,5]. Their affinity for progesterone receptors is comparable with that of progesterone. 5-Reduced pregna-D'-pentarane derivatives are analogues of 5α/5β-progesterone metabolites. Progestogen activity of 5-reduced progesterone metabolites is much lower than that of the initial hormone: 5α-dihydroprogesterone derivatives only partially retain, while 5β-dihydroprogesterone metabolites completely lose their hormonal activity, which correlates with the affinity of these compounds for progesterone receptors [3]. These data suggest that 5α/β-reduction of progesterone analogues having additional D' cycloalkane rings would produce similar changes. The Clauberg—McFeil test showed that 16α,17α-cyclohexano-5α/β-dihydroprogesterones possess no progestogen activity [1]. Both compounds are potent antigestogens inhibiting progesterone-induced stimulation of endometrial cell proliferation. 5α-Reduced analogues counteract the pregnancy-maintaining effect of progesterone. It should be emphasized that the effective dose of 5α/β-reduced pregna-D'-pentaranes is comparable with that of the natural hormone [8]. We hypothesize, that either reduction of A rings in pregna-D'-pentaranes does not decrease their affinity for progesterone receptors or these compounds act by a nonclassical pathway. The protein isolated from soluble fractions of rat uterus and specifically binding pregna-D'-pentaranes is probably involved in this tentative pathway [10]. Probably, there are some specific receptors for 5-reduced pregna-D'-pentaranes.

The interaction of 16α,17α-cyclohexano-5α/β-dihydroprogesterones with soluble uterine fraction proteins was studied by competitive assay and direct binding of ^{3}H-labeled compounds.

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MATERIALS AND METHODS

We studied 1,2,6,7-^{3}H-progesterone (specific radioactivity 86 Ci/mmol, St. Petersburg), 6α-methyl-1,2-^{3}H-

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16α,17α-cyclohexanoprogesterone (43 Ci/mmol), 1,2,3',4'-^{3}H-16α,17α-cyclohexano-5α-dihydroprogesterone (83 Ci/mmol), and 1,2,3',4'-^{3}H-16α,17α-cyclohexano-5β-dihydroprogesterone (83 Ci/mmol) [9]. Unlabeled compounds were obtained as described previously [8]. Other reagents were from Sigma.

Experiments were performed on adult female rats weighing 180-200 g. The animals were intramuscularly injected with 1 μg estradiol in 0.2 ml propylene glycol for 4 days. The rats were decapitated 1 day after the last injection. The uteri were removed and homogenized in 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 30% glycerol. All procedures were performed at 0-4°C. The supernatant (cytosolic fraction) containing 5-8 mg/ml protein was obtained by centrifugation of the homogenate at 50,000*g* for 1 h. Aliquots of the cytosolic fraction (100 μl) were incubated with 3-4 mBq ^{3}H-progesterone (in the presence of 3 μM hydrocortisone) and unlabeled competitors in concentrations of 0-10 μM for 20 h to study the interaction of test compounds with progesterone receptors. ^{3}H-6α-Methyl-16α,17α-cyclohexanoprogesterone (6 mBq) was used as a labeled ligand to study the interaction of steroids with the protein specifically binding pregna-D'-pentaranes. The reaction was performed in the presence of 127 nM progesterone inhibiting binding with progesterone receptors and varying concentrations of competitors. Direct interaction of ^{3}H-16α,17α-cyclohexano-5α/β-dihydroprogesterones (4-6 mBq) with proteins was studied in the presence of unlabeled steroids in various concentrations. Protein-bound and free ligands were separated with dextran-coated activated charcoal for 5 min. After centrifugation, radioactivity in supernatant aliquots was measured with an efficiency of 20%. This method and calculations of the equilibrium dissociation constant (C_d) and relative competitive activity (RCA) were described elsewhere [4,5]. Protein content was measured by Coomassie staining [7].

RESULTS

5-Reduced progesterone analogues weakly interact with progesterone receptors and pregna-D'-pentarane-binding protein (Table 1, Fig. 1). C_d for these compounds surpassed 1 μmol, which was 2.5 and 1.5 orders of magnitude higher than that for the corresponding high-affinity protein ligands. It should be emphasized that 5β-reduced derivative had greater C_d in relation to 2 proteins compared to that of 5α-reduced analogue, which indicated similar structure of steroid-binding sites in both proteins. Direct binding of ^{3}H-labeled 16α,17α-cyclohexano-5α/β-dihydroprogesterones by soluble uterine fraction proteins was characterized by C_d 0.2 and 2.3 μmol for 5α- and 5α/β-analogues, respectively. These results are in contradiction with the assumption that rat uterus contains specific high-affinity soluble receptors for 5α/β-analogues. Experiments on rabbits demonstrated high antiprogestogen activity of 16α,17α-cyclohexano-5α/β-dihydroprogesterones [8]. It cannot be excluded that gestogen receptors in rabbits differ from those in rats. In special experimental series we studied soluble uterine

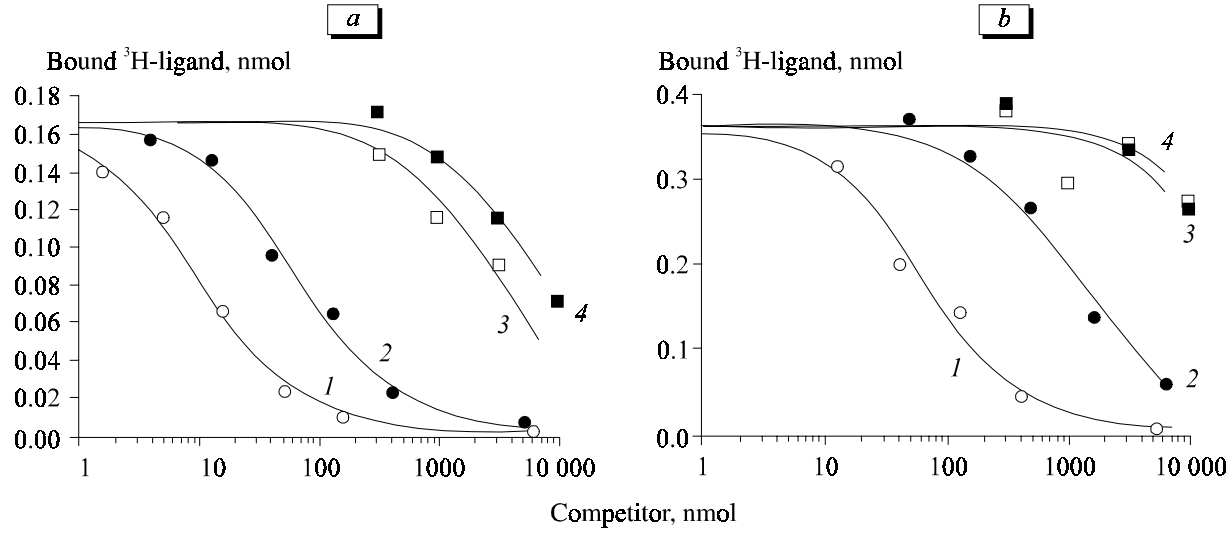

Fig. 1. Competitive assay of interaction between 5-reduced 16α,17α-cyclohexanoprogesterone analogues and progesterone receptor (*a*) or protein from soluble rat uterine fraction specifically binding pregna-D'-pentaranes (*b*). *a*) progesterone (*1*), 16α,17α-cyclohexano-6α-methylprogesterone (*2*), 16α,17α-cyclohexano-5α-dihydroprogesterone (*3*), and 16α,17α-cyclohexano-5β-dihydroprogesterone (*4*). *b*) 16α,17α-cyclohexano-6α-methylprogesterone (*1*), progesterone (*2*), 16α,17α-cyclohexano-5α-dihydroprogesterone (*3*), and 16α,17α-cyclohexano-5β-dihydroprogesterone (*4*).

TABLE 1. Interaction of 5-Reduced 16α,17α-Cyclohexanoprogesterone Analogues with Progesterone Receptor (I) and Protein from Soluble Rat Uterine Fraction (II) Specifically Binding Pregna-D'-Pentaranes

^{3}H-ligand/protein	Unlabeled competitor	C_d, nmol	RCA
Progesterone/I	Progesterone	4.8±1.1 (3)	1
	16α,17α-Cyclohexano-6α-methylprogesterone	36.0 (2)	0.167
	16α,17α-Cyclohexano-5α-methylprogesterone	1790±460 (3)	0.0030±0.0009
	16α,17α-Cyclohexano-5α-dihydroprogesterone	7380±3940 (3)	0.0012±0.0006
16α,17α-Cyclohexano-6α-methylprogesterone/II	16α,17α-Cyclohexano-6α-methylprogesterone	56.9±31.9 (3)	1
	Progesterone	1100±595 (3)	0.046±0.008
	16α,17α-Cyclohexano-5α-dihydroprogesterone	7940±5130 (3)	0.027±0.014*
	16α,17α-Cyclohexano-5β-dihydroprogesterone	345000±327000 (3)	0.0014±0.0009

Note. Number of measurements is shown in parentheses. *p<0.05 compared to 5β-analogue.

fraction from estrogenized rabbits. 5-Reduced derivatives had low affinity for progesterone receptors: C_d for 16α,17α-cyclohexano-5α- and 5β-dihydroprogesterone were 1 and 0.68 μmol, respectively; RCA (compared to progesterone) were 0.0179 and 0.0226, respectively. Two experiments with rabbit samples and ^{3}H-16α,17α-cyclohexano-5β-dihydroprogesterone as a labeled ligand showed that C_d and RCA were 590 nmol and 0.06, respectively. Thus, 16α,17α-cyclohexano-5α/β-dihydroprogesterones probably do not have high-affinity soluble receptors in the uterus of estrogenized rats and rabbits. High biological activity of these compounds probably results from their low clearance due to low-intensity metabolism and/or strong binding to blood transport proteins. It can not be excluded that the effects of these substances are realized via extragenomic membrane receptors, whose hormonal specificity differs from that of the classic soluble gestogen receptors [6]. It should be emphasized that RCA of 5-reduced pregna-D'-pentaranes in relation to progesterone receptors in the uterus of pregnant rats [2] far surpassed the values obtained in our experiments. This contradiction is probably related to peculiarities of physiological regulation of receptor properties, *e.g.*, their phosphorylation.

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REFERENCES

1. A. V. Kamernitzky and I. S. Levina, *Khim.-Farm. Zh.*, **25**, No. 10, 4-16 (1991).
2. E. N. Kareva, A. V. Kamernitzky, I. S. Levina, *et al.*, *Eksp. Klin. Farmakol.*, **62**, No. 5, 25-27 (1999).
3. V. B. Rozen and A. N. Smirnov, *Receptors and Steroid Hormones* [in Russian], Moscow (1981).
4. A. N. Smirnov, E. V. Pokrovskaya, V. P. Shevchenko, *et al.*, *Biokhimiya*, **61**, No. 9, 1279-1287 (1998).
5. A. N. Smirnov, E. V. Pokrovskaya, V. P. Shevchenko, *et al.*, *Bioorg. Khimiya*, **25**, No. 10, 774-781 (1999).
6. P. F. Blackmore, J. F. Fisher, C. H. Spilman, and J. E. Bleasdale, *Mol. Pharmacol.*, **49**, 727-739 (1996).
7. M. M. Bradford, *Analyt. Biochem.*, **72**, No. 2, 248-254 (1976).
8. I. S. Levina, G. V. Nikitina, L. E. Kulikova, and A. V. Kamernitzky, *Rus. Chem. Bull.*, **44**, No. 3, 547-550 (1995).
9. V. P. Shevchenko, I. Yu. Nagaev, A. V. Potapova, *et al.*, *J. Labelled Cpd. Radiopharm.*, **41**, 919-925 (1998).
10. A. N. Smirnov, E. V. Pokrovskaya, G. S. Kogteva, *et al.*, *Steroids*, **65**, No. 3, 163-170 (2000).