# PHARMACOLOGY AND TOXICOLOGY

# Interaction of Selected Pregna-D'-Pentaranes with Cytosolic Receptors of Myometrium and Endometrium in Various Disease States

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The relative capacities of several pregna-D'-pentaranes to combine with cytosolic receptors of the myometrium and endometrium in various disease states are investigated, and one of the compounds, 6α-methyl-16α,17α-cyclohexanoprogesterone, is identified as holding particular promise for clinical use in replacement therapy and as a contraceptive agent.

**Key Words**: *endometrium; myometrium; tumors; pregna-D'-pentaranes*

Synthetic progestins are widely used in the combined modality treatment of estrogen-dependent tumors [2,3,5]. The quest for long-acting progestins with heightened activity has led to the production of pregna-D'-pentaranes - a new class of modified gestagens obtained (in the Laboratory for Chemistry of Corticoid Compounds, N. D. Zelinskii Institute of Organic Chemistry, Moscow) by introducing into the progesterone molecule an additional carbocycle condensed with the steroid skeleton in the 16α and 17α positions.

In the study reported here, several compounds of this class (Fig. 1) showing high gestagenic activity were tested for their relative abilities to bind to cytosolic progesterone receptors (cPR) of uterine tissues.

## MATERIALS AND METHODS

Materials used in this study were myometria (tissues from myomatous nodes, histologically unchanged myometria from patients with myoma, and myometria from patients with prolapsed uterus) and endometria (glandular-fibrous and glandular-cystic polyps and highly, moderately, and slightly differentiated adenocarcinomas removed during surgery). These tissues were reduced to powder in liquid nitrogen and then extracted with 7-8 volumes of TED buffer, pH 7.4, containing 10 mM Tris-HCl (Merck), 1.5 mM EDTA (Sigma), 0.5 mM dithiothreitol (Uoch-Light Laboratorie), and 0.3% sodium azide (Merck), supplemented with 10% glycerol of analytical grade. The homogenates were centrifuged for 15 min at 1500 *g* and 0-4-C. From the supernatants, cytosol was obtained by centrifugation at 105,000 *g* for 60 min at 0-4°C. Relative binding capacities of the pentaranes were determined by the conventional procedure [6] with the following modifications. To each test tube prepared for incubation, 5 nM of $^3$H-progesterone, the test pentarane in concentrations from $10^{-9}$ to $10^{-3}$ M, and 100 μl of cytosol with a cPR concentration of at least 50 fmol protein per mg were added, the mixture was incubated for 2 h, and then, after adding 100 μl of TED buffer (containing 50% glycerol), subjected to a further 2-h in-

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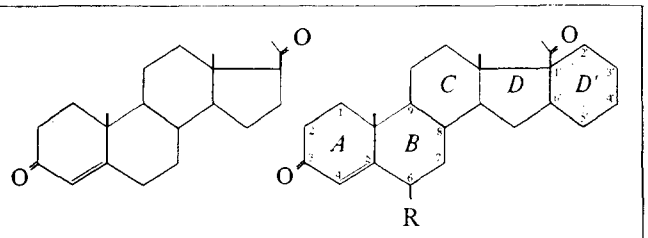
cubation at 0-4°C. Thereafter, the unbound hormone was precipitated with a suspension of Dextran T-70(Serva)-coated activated charcoal Norit A (Serva) and centrifuged at 1500 g for 15 min at 0-4°C. Next, 200 μl of supernatant from each tube was transferred to a flask containing 5 ml of dioxane-based scintillation fluid. Radioactivity was measured in an SL-30 liquid scintillation radiometer (Intertechnique), and relative binding capacities of the pentaranes were calculated by the formula: [P]/[I], where [P] is the progesterone concentration in the system and [I] is the pentarane concentration at which 50% displacement of progesterone from its receptor binding sites is observed. Protein concentration was determined by Lowry's method [4].

## RESULTS

Relative binding capacities of the tested pregna-D'-pentaranes with the gestagen-binding system of human myometrial and endometrial cytosols in different disease states are shown in Table 1.

Compound I exhibited a fairly strong affinity for cPR of the histologically unchanged myometrium, but failed to displace progesterone from its receptors in myomatous node tissues. The relative capacity of this compound for binding to cPR of tissues taken from prolapsed uteri of postmenopausal women was lower by a factor of almost 2 than for binding to cPR of unchanged myometrial tissues from women of reproductive age. Its affinity was highest for cPR of unchanged myometrial tissue and appreciably lower for those of all pathologically changed myometrial tissues.

As regards endometrial tissues, compound I exhibited slight affinity for cPR of tissues from highly or moderately differentiated adenocarcinomas and a complete lack of affinity for those of all other tissues used.




Progesterone I-V Pentaranes

| Compound | Double bond | R |
|---|---|---|
| *I* – 16α, 17α–cyclohexanoprogesterone | – | H |
| *II* – 6α–methyl–16α, 17α–cyclohexanoprogesterone | – | $CH_3$ |
| *III* – 6α–methyl–16α, 17α–cyclohexano–6–denydroprogesterone | $\Delta^6$ | $CH_3$ |
| *IV* – 16α, 17α–cyclo-hexano–6–denydroprogesterone | $\Delta^6$ | H |
| *V* – 16α, 17α–cyclohexanoprogesterone | $\Delta^{3,5}$ | H |

Introduction of a methyl group in position 6α of the base pentarane (compound II) has been reported to result in enhanced gestagenic activity [1]. In our study, compound II was more effective than the other pentaranes in displacing progesterone from its cPR binding sites of uterine tissues. Its relative binding capacity was highest with histologically unchanged myometrium and somewhat lower with myometrial tissues from prolapsed uteri. With myomatous node tissues, this capacity was only about one-third that with histologically unchanged myometrium. Thus, compound II, like compound I, showed the highest affinity for the latter tissue.

Compound II also bound with high affinity to cPR of the glandular-fibrous and glandular-cystic polyps and with lower affinity to those of

**TABLE 1.** Relative Binding Capacity of Pregna–D'–Pentaranes for the Cytosolic Gestagen–Binding System of Pathologically Changed or Unchanged Human Myometrium and Endometrium

| Compound | Myometrium | | | Endometrium | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | polyps | | adenocarcinomas | | |
| | prolapsed uterus | histologically unchanged | myomatous node | glandular-fibrous | glandular-cystic | highly differentiated | moderately differentiated | slightly differentiated |
| I | 2.70 | 5.00 | 0.00 | 0.00 | 0.00 | 0.71 | 0.03 | 0.00 |
| II | 22.4 | 25.85 | 6.83 | 11.6 | 0.00 | 2.70 | 0.00 | 1.58 |
| III | 0.00 | 1.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| IV | 0.00 | 1.41 | 3.98 | 5.00 | 6.23 | 8.90 | 1.26 | 0.80 |
| V | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 3.16 | 3.27 |

adenocarcinoma cells regardless of the stage of tumor differentiation. Its activity decreased in the order glandular-fibrous polyps>glandular-cystic polyps>highly differentiated adenocarcinomas> moderately differentiated adenocarcinomas>slightly differentiated adenocarcinomas.

Compound III, obtained by introducing an extra double bond in position 6, proved to be the least active among the tested pentaranes and exhibited affinity (rather slight) only for cPR of histologically unchanged myometrium.

Introduction of both a methyl group and an extra double bond in position 6 resulted in a compound (compound IV) with characteristics somewhat different from those of the preceding three compounds. Thus, it displayed a higher affinity for cPR of myomatous node tissues than for cPR of histologically unchanged myometrium and did not displace progesterone from ligand-receptor complexes in myometrial tissues of prolapsed uteri. Compound IV, therefore, was unique in showing enhanced affinity for cPR of neoplastically transformed myometrium. This pentarane was capable of competing with progesterone in all of the pathological states of the endometrium studied. Its relative binding capacity was high in endometrial polyps and highly differentiated adenocarcinomas and much lower in slightly or moderately differentiated adenocarcinomas.

Compound V, obtained through introduction of an extra double bond directly into the carboxyl D' in position 3', exhibited only low affinity for cPR of myomatous node tissues but displaced progesterone from its receptor binding sites in tissues of slightly and moderately differentiated adenocarcinomas. Thus, chemical modification of the base pentarane resulted in a compound which is capable of displacing progesterone from cPR exclusively in neoplastically transformed tissues of human uterus.

Because of their marked ability to displace progesterone from its receptor sites, three of the compounds (II, IV, and V) may be recommended for further study as candidates for clinical use in hormonal therapy of uterine tumors.

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