PHARMACOLOGY AND TOXICOLOGY

Relative Binding Capacity of Pentarane Substances with Plasma Membrane Receptors of Uterine Cells of Oophorectomized Rats

P. V. Sergeev, A. V. Kamernitskii, I. S. Levina, N. Yu. Tkacheva, and E. N. Kareva

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The relative capacity of substances of the pentarane group for binding with progesterone receptors of the plasma membranes of uterine cells of oophorectomized rats is studied. Introduction of an extra carbocycle D' in the progesterone molecule at the 16α and 17α sites and further modification of the molecule cause an increase of the relative binding capacity of these compounds. Analysis of the findings identifies substance III, showing the highest activity toward all the discussed parameters and a promising candidate for further preclinical studies.

Key Words: *pentaranes; plasma membrane progesterone receptors; rat uterus*

The search for new effective agents for hormone therapy in obstetrics and gynecology has stimulated goal-directed synthesis of gestagens. Transformation of progesterone molecules has led to the creation of a series of synthetic steroids with a more expressed dissociation of the properties intrinsic to the natural hormone [4,7].

The insertion into progesterone molecules of an extra carbocycle D' condensed with the steroid skeleton in the 16α- and 17α-positions has given rise to a new class of highly active progestins known as pregna-D'-pentaranes [3].

A comparative study of the interactions between progesterone and some of its pentarane derivatives with the gestagen-binding sites of rat uterine cytosol revealed that the relative ability of pentaranes to bind with the cytosol receptors of progesterone (RBA_{cyt}) is quite poor and does not correspond to the high gestagen activity of the agents *in vivo* [5].

Since the receptor system of the progesterone target cell has, besides the cytosol, a plasma membrane component [6,11], we thought it expedient to study the relative binding ability of pentarane derivatives with the gestagen-binding sites of plasma membranes (RBA_{pm}) of uterine cells in oophorectomized rats to detect possible correlations with their biological activity.

MATERIALS AND METHODS

Experiments were carried out with 46 female rats weighing 120-140 g, 6 to 8 animals per group. For studies of the uterotropic activity, an oil solution of pentaranes in a dose of 1 mg/100 g body weight was injected intraperitoneally on day

Department of Molecular Pharmacology and Radiobiology, Biomedical Faculty, Russian State Medical University, Moscow

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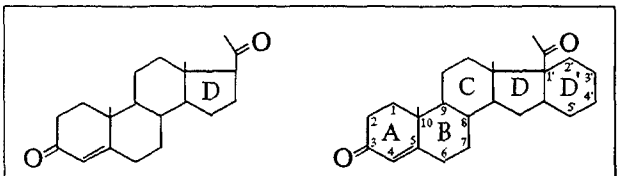

I-progesterone II-IV - pentaranes

Compound	Double bound	R
II – 16α–, 17α–cyclohexaneprogesterone	–	H
III – 6α–methyl–16α–, 17α–cyclohexaneprogesterone	–	CH_3
IV – 6α–methyl–16α–, 17α–cyclohexane–6–dehydroprogesterone	Δ^6	CH_3
V – 16α, 17α–cyclohexane–6–dehydroprogesterone	Δ^6	H
VI – 16α–, 17α–cyclohexaneprogesterone	$\Delta^{3,4}$	H

Fig. 1. Chemical structure of progesterone and pentaranes.

4 after oophorectomy [2]. After being weighed, the animals were decapitated under ether anesthesia 24 h after drug injection. The uteruses were freed of fatty and connective tissue and weighed. Ute-rotropic activity (UA) was estimated from the formula:

$$UA = (\text{uterine mass/rat mass}) \times 100\%.$$

For estimation of the RBA_{pm}, plasma membranes were isolated [9] and the relative binding ability was measured after Scheider [13]. Protein was measured after Lowry in Peterson's modification [10].

Results were statistically processed using Student's *t* test [12].

RESULTS

Pentaranes were prepared at the Department of Chemistry of Corticoid Compounds of the N. L. Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences. Their chemical structure is presented in Fig. 1 [3].

The results of analysis of the relative binding ability of the new agents with progesterone-binding sites of plasma membranes of rat uterine cells are presented in Table 1. The data indicate that on the whole pentaranes effectively expel progesterone from its binding sites in the plasma membrane and show a high UA.

A detailed analysis of the specific binding activity of pentaranes, making use of additional data, helped to divide these agents tentatively into three groups; 1) compound III, which proved to be the most active; besides a high RBA_{pm} value (12.5%) and UA (9.5%), it is characterized by a 100% contraceptive effect, a high capacity for preserving pregnancy (92%), and the highest value in the Clauberg-McFeil test among the tested pentaranes [1,8]; 2) substances II and IV may be referred to the second group; they are characterized by mean values of the studied parameters and lower (in comparison with substance III) contraceptive activity, ability to preserve pregnancy, and values in the Clauberg-McFeil test [1]; 3) this last group includes substances V and VI with the lowest RBA_{pm} values (Table 1).

Hence, introducing into the progesterone molecule an extra carbocycle D' in the 16α and 17α positions boosts the specific activity of this substance (substance II). Further modification of the molecule via the introduction of a methyl group in position 6 (substance III) causes a still more marked increase of both its gestagen activity and other specific parameters. However, the introduction of an additional double bond in position 6 (substances IV and V) markedly lowers the examined parameters in comparison with those of the parent pentaranes (substances III and II, respectively) [1].

The introduction of a double bond in the carbocycle D' (substance VI) completely abolishes its ability to compete for membrane receptors with progesterone. It is interesting that the same compound shows the lowest activity (0.023) in the Clauberg-McFeil test among the examined substances [1].

Analysis of the specific activity of these new pentarane agents helped identify the substance characterized by the highest biological activity, namely, substance III, which is a promising candidate for further studies and clinical use in replacement therapy and as a contraceptive.

TABLE 1. Relative Binding Ability of D' Pentaranes with Progesterone Receptors of Plasma Membranes of Rat Uterine Cells and their Uterotropic Activity ($M \pm m$)

Agent	RBA_{pm}, %	UA, %
I	100.0	5.26±1.276
II	3.794	8.39±0.5*
III	12.589	9.5±1.09*
IV	7.996	9.5±0.27*
V	0.501	8.48±0.27*
VI	0.0	6.74±1.34

Note. Asterisk: $p<0.05$ vs. agent I.

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Comparative Analysis of Progesterone and Estradiol Reception in Human Myoma

P. V. Sergeev, E. N. Kareva, and N. Yu. Tkacheva

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Cytosolic and plasma membrane receptors for progesterone and estradiol are studied in myomatous nodes (MN) and in histologically unaltered myometrium (HUM) against the background of myoma. The level of cytosolic receptors for both hormones is higher in the myoma cells than in the essentially healthy myometrium. In the plasma membranes the progesterone reception is reduced and the estradiol reception is unchanged compared with HUM.

Key Words: *progesterone receptors; estradiol receptors; plasma membrane; myoma*

The role of steroid hormones in the formation and development of certain tumors has been studied for a long time. The presence of steroid receptors, their number, and the tissue ratio are fundamental for the diagnosis and choice of therapy in a number of oncological diseases. At the same time, the presence of cytoplasmic and nuclear receptors for sex steroids and their characteristics are by no means always indicative of the hormonal dependence of a pathological process [2,7,11]. New data are being published on the structure of receptors of the cells that are targets for steroid hormones; specific binding sites for steroids have been found on the plasma membranes (PM) [5,6], mitochondria [4], and lysosomes [10]. We have shown that the PM of human endometrial and myometrial cells has specific binding sites for 17β-estradiol; these binding sites have a high affinity for the ligand and change their characteristics according to the stage of tumor growth [3]. Since the relative plasma content of estradiol and progesterone, rather than the absolute concentration of estrogens [1,8],

Department of Molecular Pharmacology and Radiobiology, Biomedical Faculty, Russian State Medical University, Moscow

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