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Bimodal binding and free energy of the progesterone receptor in the induction of female sexual receptivity by progesterone and synthetic progestins

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ABSTRACT

Synthetic progestins (SPs) are used for regulation of fertility, contraception and hormone replacement therapy. The acetylated medroxyprogesterone (MPA), megestrol (MGA) and chlormadinone (CLA) are related to progesterone (P). Other SPs are 19-nortestosterone derivatives such as: norethisterone (NET), norethynodrel (NED) or the 13-ethyl gonane, levonorgestrel (LNG). We studied MPA, NET, NED and LNG in a dose–response manner to induce sexual receptivity in rats. Results showed that MPA, NET and NED act as partial agonists, with similar or lower potency than P. However, LNG is a full agonist. Additionally, the molecules of MPA, MGA, CLA, NET, NED, LNG, and P, were submitted to computer calculations at *ab initio* quantum mechanics theory, to obtain their electronic structure and molecular properties. The aim was to correlate their behavioral effect with their physicochemical properties. In addition, the crystals of P, NET and LNG bound to the progesterone receptor (PR) were studied. The PR crystallizes as a dimer forming two monomers (mA and mB), in which Gln725 interacts in either of two possible ways with the C3-carbonyl pharmacophore of progestins. P binds differentially to both PR monomers, while NET binds exclusively as mA and LNG binds only as mB in both monomers with no difference. Energetically, binding of LNG and P to mB, is more favorable than that of NET and P to mA. Consequently, this bimodal mechanism increases the action possibilities of SPs on biological systems. Interestingly, progestin potency depends mostly on local molecular structure and electronic features, prevailing over total molecular properties.

1. Introduction

Synthetic progestins (SPs) are compounds related chemically with pregnane, androstane, or estrane structures, used primarily as contraceptives and for hormone replacement therapy. The history of SPs starts in the 1950s, when Carl Djerassi et al., synthesized the estrane derivative norethisterone, also called norethindrone [1]. Norethynodrel, another estrane-like compound, was also developed in the early 50s [2]. Those compounds, in addition to lacking carbon 19, have 17β-hydroxyl and 17α-ethynyl groups.

Some SPs such as the acetylated ones, *e.g.*: medroxyprogesterone (MPA), megestrol (MGA) and chlormadinone (CLA), are structurally and chemically related to progesterone (P). By contrast, the group formed by NET, LNG and NED have significant differences with P, mostly at the C17 region, though they maintain the C3-carbonyl group in the A-ring. Those compounds are also 19-nor, a feature giving some flexibility to the A–B ring system, and also decreasing their hydrophobicity. SPs exert their progesterone-like effect by interacting with the progesterone receptor (PR). In fact, most SPs have a larger affinity for the PR than P. Thus, binding affinity and stereochemical properties are the main reasons for their biological and pharmacological differences among them.

Progesterone has effects on psychobiological processes such as female sexual behavior. In rodents, for instance, P induces the lordosis reflex, along with attractive and proceptive behavior [3]. Our group has reported the potencies of P, MGA and CLA for inducing sexual behavior in estrogen-primed rats [4]. MPA has also been found to promote sexual receptivity in estrogen-primed rats [5].

In this work we explored the capacity to induce sexual behavior of P, NET, NED, LNG, MGA, CLA, and MPA using dose–response curves. The aim was to establish a correlation between the chemical structure and physicochemical properties of progestins with their potency to induce sexual receptivity and proceptivity. Thus,

Abbreviations: SPs, synthetic progestins; MPA, medroxyprogesterone acetate; MGA, megestrol acetate; CLA, chlormadinone acetate; NET, norethisterone; NED, norethynodrel; LNG, levonorgestrel; PR, progesterone receptor; mA, monomer A; mB, monomer B; EB, estradiol benzoate; LQ, (number of lordosis/10 mounts) × 100; LS, lordosis score; EP, electrostatic potential; RMSD, root mean square deviation.

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a quantum mechanics analysis was used to compare the electronic properties of progesterone and the six SPs. Steroid binding characteristics and free energies were also analyzed from the crystals of P, NET and LNG bound to the PR.

2. Materials and methods

2.1. Animal care

Sexually inexperienced adult Wistar female rats (200–250 g, body weight) bred in our colony were used. Animals were housed in groups of four per cage inside a vivarium with 12 h light:12 h dark (lights on at 22:00 h) and ambient temperature of $23 \pm 2\,^{\circ}C$. Purina rat chow and water were available *ad libitum*.

2.2. Surgery

Two weeks before initiating behavioral experiments rats were ovariectomized bilaterally under Xilazine-Ketamine anesthesia.

2.3. Steroids

Estradiol benzoate (EB), and four synthetic progestins, one of them related chemically to P, namely: medroxyprogesterone acetate (MPA; 6α-methyl-3,20-di-oxopregn-4-en-17-yl acetate) were used. The other three SPs were: 19-nor, 17β-hydroxy,17α ethynylated, that is: norethisterone (NET; 17α-ethynyl-19-nor-4-androsten-17β-hydroxy, 3-one); norethynodrel (NED, 17β-hydroxy-19-nor-17(-pregn-5(10)-en-20-yn-3-one) and levonorgestrel (LNG; 13-β-ethyl-17α-ethynyl-17β-hydroxygon-4-en-3-one). The steroids were dissolved in carthamus oil and injected subcutaneously (0.2 ml), all purchased from Sigma (St. Louis, MO).

2.4. Treatment

Two weeks after ovariectomy females received a sc injection of 2 μg EB. Forty two hours later animals received a single progestin injection. Six dose levels were used for each progestin: 0.66, 3.30, 16, 80, 400 and 2000 μg.

2.5. Testing procedures

Sexual receptivity and proceptivity were determined by placing the females in a circular Plexiglas arena with a sexually vigorous male. Receptivity was quantified by using the lordosis quotient: LQ = (number of lordosis/10 mounts) × 100. The intensity of lordosis was determined by the lordosis score (LS), as described by Hardy and De Bold [6]. Thus, the intensity of each lordosis was measured in a scale ranging from 0 to 3. The incidence of proceptive behaviors (hopping, darting and ear wiggling) was also determined. A rat showing any of those behavioral responses was considered proceptive.

2.6. Statistical analysis of behavioral responses

Relative potencies of the tested progestins for inducing lordosis were calculated according to the procedure described by Tallarida and Murray [7]. This method allows the calculation of regression lines, analysis of parallelism, determination of a common slope, and calculation of the potency of each progestin in relation to P, which is the reference drug with a relative potency = 1. Parameters for considering significant parallelism among the various curves was established at a 95% confidence level.

The method of Tallarida and Murray [7] was also used to determine the relative potencies of SPs for comparing the magnitude of the LS and for inducing proceptivity through a quantal dose–response relationship. The procedure is similar to that described above except that, in this case, the values used were not the original values but, rather, the corresponding probit values (probability units).

2.7. Molecular assessment

The aim was to obtain the lowest energy conformation and physicochemical properties of the steroids using *ab initio* quantum mechanics theory. Geometry optimization calculations, started by applying the semi-empirical method PM3, followed by the low level model Hartree–Fock STO-3G*, then, 3-21G* and finally with 6-31G* basis set level. The full procedure has been published elsewhere for progesterone [8] and NET [9]. Thus, for all progestins were calculated: total energy, solvation energy, dipole moment, surface, volume and polar surface area. The electrostatic potential and atomic charges were also defined. The used software was: Spartan'08 [10] for calculations and the program WebLab ViewerPro [11] for additional molecular graphics.

2.8. Steroid-receptor interaction analysis

The crystals of Progesterone, NET and LNG, complexed to the progesterone receptor (PDB ID: 1A28, 1SQN and 3D90, respectively), were retrieved from the PDB source [12]. The interaction between the steroid and the adjacent residues at 4 Å from the PR, were analyzed with the Ligand Explorer program [13]. The analysis was focused on binding interactions such as: hydrogen-bonds, water bridged H-bonds and hydrophobic interactions. The number and type of binding interactions and the calculation of the Gibbs free energy of each complex were used as an index of binding capacity. The free energy calculation started with the C3-carbonyl group of NET, LNG and P, bound to Gln725, Arg766 and the associated water molecule of the PR. Likewise, the energy profile of the Cβ-Cγ-Cδ-N dihedral angle rotation of Gln725 was calculated. Next, we obtained the free energy of the progesterone receptor-progestin complex, to assess to some extent the energy of the receptor ligand interaction. In order to include only the first core of residues belonging to the PR, the cut off distance between the progestin and the receptor was set at 4 Å. This included 12–14 amino acids and 22–32 total interactions.

A simplified procedure to calculate the free energy is given by the formula:

$$E = [AP] - [A + P]$$

where

E = free energy.
$[AP]$ = energy of the complex, formed by the receptor amino acids and the progestin.
A = energy of the amino acids.
P = energy of the progestin.

All molecular energies were determined by single point calculations using the Hartree–Fock theory at the 6-31G* basis set level.

3. Results

3.1. Behavior experiments

Dose–response curves showed some important differences among them. The MPA curve was markedly similar to that of P: the ED_{50} is the same for both (68 μg). MGA, however, had a higher ED_{50} (212 μg) than P and MPA. The lordosis response increased