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The importance of the chemical structure of pregnanes in the concurrent inhibition of estrous behavior in the female rat

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We dedicate this work to our dear friends and professors Carlos Beyer and Richard E. Whalen.

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ABSTRACT

An investigation of aspects ranging from behavior to molecular electronic structure and physicochemical properties was performed to explore the role of 5α-pregnanedione (5α-DHP), 5β-pregnanedione (5β-DHP) and their precursor progesterone (P) on the concurrent inhibition of the sexual lordosis response in female rats. The concurrent inhibition of lordosis behavior occurs when ovariectomized rodents are primed simultaneously with estradiol (E2) and P. Thus, a second administration of P 40 h later fails to induce the expected sexual response that takes place when E2 and P are administered sequentially 40 h apart. In this study, it is hypothesized that the modulation of the sexual behavior display depends to some extent on the molecular structure and associated physicochemical properties of steroid hormones such as P and its metabolites. Therefore, these molecules must be studied chemically and structurally to explain their role in sexual behavior, including the concurrent inhibition effect. Analysis of the electronic structure and physicochemical properties demonstrated striking differences in the A-ring region of P, 5α-DHP and 5β-DHP, particularly in atomic charges, dipole moment (DM) and electrostatic potentials. Similarly, the structural differences between the *trans* (5α-DHP) and *cis* (5β-DHP) configurations were remarkable. 5α-DHP most significantly promoted the concurrent inhibition of the lordosis behavior, followed by P and 5β-DHP. These data indicate that variations in pregnane structure are related to the extent of the concurrent inhibition effect and also suggest that P may act as a prehormone in certain functions of the central nervous system.

1. Introduction

The chronological administration of estrogens and progesterone (P) in the ovariectomized (ovx) rat, which promotes a sexual response resembling the natural estrus cycle, was shown long ago by Boling and Blandau [1]. Those authors established clearly that estrogen priming followed by progesterone administered after an interval of approximately 40 h induced a display of sexual behavior in the spayed rat. Later, the estrus behavior patterns, consisting of three main events, namely attractivity, proceptivity and receptivity, were described by Beach [2]. The male is strongly attracted by the female, who executes stereotyped movements such as hopping, darting and ear-wiggling, which are the typical reactions that form proceptive behavior. Finally, the receptivity that allows the male to mount the female occurs because of the lordosis reflex, which includes lowering of the dorsoposterior region, forming a concavity and simultaneously raising the head, the rump and the tail.

Notably, when a high dose of P is administered near the time of the priming estradiol (E2) injection, an inhibition of the estrus behavior is observed. Thus, a second injection of P 40 h later fails to elicit the expected lordosis response; this effect, called concurrent inhibition, was denominated by Powers and Moreines [3]. The interval of time from P administration to achieve concurrent inhibition of estrus induction by E2 ranges from 16 h before E2 priming to 20 h after when P is administered in low doses [4]. This lapse of 36 h is sufficiently long for P conversion, likely

Abbreviations: 5α-DHP, 5α-pregnanedione or 5α-dihydroprogesterone; 5β-DHP, 5β-pregnanedione or 5β-dihydroprogesterone; P, progesterone; ovx, ovariectomized; E2, estradiol; EB, estradiol benzoate; DM, dipole moment; ANOVA, analysis of variance; CNS, central nervous system; sc, subcutaneous; icv, intracerebroventricular; LQ, lordosis quotient; RMSD, root mean square deviation; PR, progesterone receptor.

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yielding some derivatives capable of producing the concurrent inhibition effect. However, certain direct metabolites that result from changes in carbon 17 and the oxygen atom of the 20 carbonyl group, such as 17α-hydroxy-progesterone, 20α-hydroxy- and 20β-hydroxy-pregnenone, fail to produce concurrent inhibition in the guinea pig [5], suggesting that biochemical changes in the C17β-acetyl side-chain of the P molecule are not linked to the concurrent inhibition of the sexual behavior display.

An alternate and important metabolic route of P is the reduction of the C4-C5 π-bond of the A-ring steroid system because the activity of 5-reductases is favored by the frontier orbitals of P located in that bond [6], directly yielding two 5-reduced metabolites: 5α-dihydroprogesterone (5α-DHP) and 5β-dihydroprogesterone (5β-DHP). Notably, P is synthetized in the glia cells under the effect of estradiol [7]. Therefore, P may be converted by Δ4-3-ketosteroid-5α-(5β) reductases to 5α-(5β) pregnanediones, which may be converted afterwards to pregnanolones by the 3α-hydroxysteroid oxidoreductase enzyme [8,9].

Both pregnanediones are produced readily from P in the central nervous system (CNS). In particular, 5α-DHP is produced in the basal hypothalamus in rats [10], and 5β-DHP is synthetized in the cerebral cortex [11]. Thus, these natural progestins might be involved in certain functions of the CNS. In fact, stimulatory effects on lordosis behavior are induced by both pregnanediones and some other 5-reduced P derivatives in estrogen-primed rats. Finally, some metabolites are more potent than P at inducing the lordosis response [12,13]. The effectiveness of the 5-reduced metabolites is evident when they are implanted or administered directly in the hypothalamus [13,14], which seems to be the neural target for induce the sexual behavior display. Likewise, dose-response curves have revealed that 5α-DHP is ten times more potent than P, while 5β-DHP is equipotent to P, at inducing sexual receptivity in ovx, E2-primed rats [15].

The present study was designed to investigate the role of 5α-DHP and 5β-DHP in the concurrent inhibition of the lordosis response. P was included in the trial to improve the comparison analysis. Briefly, the study was organized in a round-robin fashion in ovx rats primed subcutaneously (sc) with estradiol benzoate (EB) and any of the three progestins, followed by a subsequent progestin intracerebroventricular (icv) administration 40 h later. Considering that 5α-DHP and 5β-DHP are isomers but have marked differences in the A-ring steroid system, they are a good model to characterize steric A-ring properties. The present hypothesis is that the steric differences in the A-rings of P, 5α-DHP and 5β-DHP may have different roles in the concurrent inhibition mechanism based on a chemical structure-biological activity relationship. To accomplish this study, computational analysis of their electronic structure and associated physicochemical properties was carried out including the precursor, P.

2. Materials and methods

2.1. Animal surgery and cannulae position

A total of 123 adult female Sprague–Dawley rats (200–270 g) bred in our colony were used and maintained on a 14-h light/10-h dark cycle schedule with the lights turned off at 10:00 a.m. The rats were ovx bilaterally, and one week later, the animals were anesthetized with xylazine (4 mg/kg) and ketamine (80 mg/kg), placed in a Kopf stereotaxic apparatus and secured with ear bars and a nose piece set at +5 mm. A 22-gauge guide cannula (Plastics One, Roanoke, VA) was implanted into the right lateral ventricle using the following coordinates: anterior–posterior +0.80 mm, mediolateral 1.5 mm, dorsoventral −3.5 mm relative to the bregma, according to the atlas of Paxinos and Watson [16]. A stainless steel screw was fixed to the skull, and both the cannula and the screw were attached to the bone with dental cement. A dummy cannula (30 gauge) was introduced into the guide cannula to prevent clogging and contamination. All of the animal experiments were in accordance with the Mexican Law for the Protection of Animals and approved by the Institutional Animal Care and Use Committee of CINVESTAV.

2.2. Steroid administration to assess the concurrent inhibition of sexual behavior

One week after the stereotaxic surgery, sexual behavior experiments were initiated to evaluate the capability of the progestins to elicit concurrent inhibition. The three progestins were tested and compared with each other in a sort of round-robin scheme. Three main groups were formed, including P, 5α-DHP and 5β-DHP groups, formed by 43, 40 and 40 animals, respectively. Additionally, 8 rats were used for the oil + estradiol benzoate control group. A total of 131 animals were injected with an initial (h 0) sc dose of 5 μg of EB dissolved in 0.1 ml sesame oil. Each of the main groups was divided into three subgroups that received concurrent administration of P, 5α-DHP and 5β-DHP, also sc. To test the concurrent inhibition effect, progestins were administered icv 40 h after the EB & progestin priming. The doses of progestins were adjusted based on the work of Czaja et al. [17] and previously published dose-response curves [15]. The steroids were purchased from Sigma (St. Louis, MO, USA). For the sake of clarity, Table 1 shows the time and dosage scheme of steroid administration for the concurrent inhibition experiment.

2.3. Behavioral tests

Behavioral trials to evaluate the concurrent inhibition effect were carried out 2 and 4 h after the icv administration of progestin

Table 1
Time course, dosage and progestin administration schedule to elicit concurrent inhibition of the sexual response.[e]

P Subgroups				5α-DHP Subgroups				5β-DHP Subgroups			
0 h (sc)[a] 4 mg & 5 μg	40 h (icv)[b]	μg	n	0 h (sc)[a] 4 mg & 5 μg	40 h (icv) [b]	μg	n	0 h (sc)[a] 4 mg & 5 μg	40 h (icv)[b]	μg	n
[P & EB]	P	130	12	[5α-DHP & EB]	P	130	12	[5β-DHP & EB]	P	130	12
[P & EB]	5α-DHP	13	9	[5α-DHP & EB]	5α-DHP	13	9	[5β-DHP & EB]	5α-DHP	13	9
[P & EB]	5β-DHP	130	10	[5α-DHP & EB]	5β-DHP	130	10	[5β-DHP & EB]	5β-DHP	130	10
[EB][c]	P	130	12	[EB][c]	5α-DHP	13	9	[EB][c]	5β-DHP	13	9
[Oil & EB][d]	Oil		8								

[a] sc, subcutaneous priming doses.
[b] icv, intracerebroventricular injection.
[c] Progestin sexual behavior control groups: EB + P; EB + 5α-DHP and EB + 5β-DHP.
[d] Oil & EB control group.
[e] An "&" denotes concurrent administration of the steroids.

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