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Research report

GnRH mediates estrous behavior induced by ring A reduced progestins and vaginocervical stimulation

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Abstract

The present study was designed to assess the participation of gonadotropin-releasing hormone (GnRH) in the display of estrous behavior induced by application of vaginal–cervical stimulation (VCS) and by the intracerebroventricular (icv) administration of progesterone and its ring A-reduced metabolites to ovariectomized (ovx), estradiol benzoate (E_2B) primed rats. Icv injection of Antide, a GnRH-1 receptor antagonist, significantly depressed lordosis behavior in ovx, E_2B-primed rats treated with icv GnRH. Application of VCS to ovx, E_2B-primed rats facilitated both lordosis and proceptivity. These behavioral responses were significantly depressed by the icv administration of Antide. Similarly, icv Antide blocked the stimulatory effect on both lordosis and proceptive behaviors elicited by progesterone and its ring A-reduced metabolites: 5α-pregnandione (5α-DHP), 5α-pregnan-3α-ol-20-one (5α,3α-Pgl) and 5β-pregnan-3β-hydroxy-20-one (5β,3β-Pgl) in ovx, E_2B-primed rats. By contrast, icv injection of Antide failed to interfere with the facilitatory effect of the synthetic progestin megestrol acetate on lordosis and proceptive behaviors. This progestin is not reduced in ring A. The results suggest that GnRH release is an important process in the chain of events leading to the display of estrous behavior in response to progesterone, its ring A-reduced metabolites, and VCS in female rats.

Keywords: GnRH; Progesterone; Megestrol acetate; 5α-Pregnan-3α-ol-20-one; 5β-Pregnan-3β-hydroxy-20-one; Antide; Lordosis; Proceptivity

1. Introduction

A well-known action of ring A-reduced progesterone (P) metabolites is the facilitation of lordosis and proceptive behaviors in estradiol (E_2)-primed rodents [7,8,33–36,38,39,41,43]. The cellular mechanism(s) by which these progestins enhance female sexual behavior is (are) unclear. The fact that concurrent administration of the classical progestin receptor (PR) antagonist RU486 abolished the estrous behavior induced by several ring A-reduced progestins [8,10,43] implicated the participation of PRs in this response. However, progestins such as 5α-pregnan-3α-ol-20-one (5α,3α-Pgl), which do not bind to the intracellular PR [70,79], induce lordosis behavior more potently than P when administered to estrogen-primed rats either intravenously or directly into the brain [7,8,39]. This suggests that ring A-reduced progestins activate the PR in a ligand independent mechanism through signaling pathways triggered at the membrane level. Indeed, several protein kinase inhibitors reduce estrous behavior induced by ring A-reduced progestins [38,39,41]. This effect (PR activation) could be produced by a direct action of the progestins at the membrane level of neurons possessing PRs or indirectly by releasing neurotransmitters or neuromodulators capable of activating intracellular signaling mechanisms in these PR neurons.

P and some of its ring A-reduced metabolites influence gonadotropin secretion by acting at the hypothalamic level. Thus, several workers using ovariectomized (ovx), E_2-primed rats found that 5α-pregnanedione (5α-DHP), 5α,3α-Pgl and 5β,3β-pregnanolone (5β,3β-Pgl) potently stimulate the release of gonadotropins by activating GnRH secretion from the hypothalamus [24,30,31,44,57,59,75,82,83]. Indeed, 5β,3β-Pgl is 1000 times more potent than P in inducing GnRH release

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both *in vivo* and *in vitro* [62,66–68]. The release of hypothalamic GnRH by ring A-reduced progestins is relevant for its stimulatory effect on lordosis behavior; as initially shown by Moss et al. [53–55], this peptide can trigger female sexual behavior in ovx or ovx-adrenalectomized rats primed with E_2 [10,21,32,42,45,73,74,81].

At least two forms of GnRH, GnRH-I and GnRH-II, are present in mammals. The presence of two GnRH isoforms suggests the existence of two cognate GnRH receptor subtypes, type 1 and 2 [51,56,76]. The GnRH type 1 receptor participates in the expression of female sexual behavior, because Antide, a type 1 receptor antagonist, blocked lordosis behavior induced by GnRH in ovx rats primed with E_2 benzoate (E_2B) [45,81].

The purpose of the present study was to use the GnRH-1 antagonist Antide to test the hypothesis that the release of GnRH participates in the facilitation of estrous behavior by ring A-reduced progestins. Because vaginal–cervical stimulation (VCS) both enhances lordosis behavior [28,37,46,48,72] and promotes GnRH release [11,16,64,78], we also tested the capacity of Antide to interfere with VCS facilitation of lordosis. The capacity of Antide to block the stimulatory effect of megestrol acetate (MA) on estrous behavior was also explored, because this progestin cannot be ring A-reduced and does not stimulate GnRH release.

2. Material and methods

2.1. Animals and surgeries

A total of 134 Sprague–Dawley female rats (240–280 g body weight), bred in our colony in Tlaxcala, were used. They were maintained under controlled temperature ($23 \pm 2\,^{\circ}C$) and light (14:10; L:D) conditions and fed Purina rat chow and water *ad libitum*. Females were bilaterally ovx under ether anesthesia and housed in groups of four. Two weeks after ovx, the females were anesthetized with xylazine (4 mg/kg) and ketamine (80 mg/kg), placed in a Kopf stereotaxic instrument (Tujunga, CA, USA), and implanted with a stainless steel cannula (22 gauge, 17 mm long; Plastics One, Roanoke, VA, USA) into the right lateral ventricle following coordinates from the atlas of Paxinos and Watson [63] (A/P + 0.80 mm, M/L −1.5 mm, D/V −3.5 mm with respect to bregma). A stainless steel screw was fixed to the skull, and both cannula and screw were attached to the bone with dental cement. An insert cannula (30 gauge) provided with a cap was introduced into the guide cannula to prevent clogging and contamination. Animal care and all the experimental procedures adhered to the Mexican Law for the Protection of Animals.

2.2. Testing procedures

Tests for sexual behavior (receptivity and proceptivity) were conducted by placing females in a circular Plexiglas arena (53 cm in diameter) with a sexually active male. The lordosis quotient [LQ = (number of lordosis/10 mounts) × 100] was used to assess receptive behavior. Proceptivity was evaluated by determining the incidence of hopping, darting, and ear-wiggling across the whole receptivity test [49]. We considered an animal proceptive when showing two of these behaviors during the testing period. This criterion was used since in our Sprague–Dawley rats only a small proportion of animals will display the three proceptive behaviors together. This may be due to the fact that our Sprague–Dawley rats rarely (<10%) show darting in our testing conditions.

2.3. Chemicals

The steroids used were: E_2B; P; 5α-DHP; 5α,3α-Pgl; 5β,3β-Pgl; and MA (17-hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate). Other drugs used were: GnRH-1 and the GnRH-1 receptor antagonist Antide [Acetyl-D-Ala(2-naphthyl)-D-Lys(*N*ε-nicotinoyl)-D-Lys(*N*ε-nicotinoyl)-Leu-Lys(*N*ε-isopropyl)Pro-D-Ala-NH_2]. Steroids and Antide were purchased from Sigma (St. Louis, MO, USA). GnRH was purchased from Peninsula Laboratories (Belmont, CA, USA). E_2B was always administered sc in 0.1 ml sesame oil.

2.4. Experiment 1

2.4.1. Effect of Antide on estrous behavior induced by GnRH-1

This experiment determined whether the lordosis behavior induced by GnRH-1 is mediated by activation of the GnRH-1 receptor. One week after implantation of a cannula in the right lateral ventricle, 19 ovx rats were primed with 5 μg of E_2B (hour 0). Thirty-nine hours later, 1 μg of Antide or vehicle (saline) in a 1 μl volume was administered intracerebroventricularly (icv). One hour after Antide or saline (40 h post E_2B), an icv injection of 50 ng of GnRH-1 was administered. The dose of Antide was taken from the study of Kauffman and Rissman [45], while the dose of the GnRH-1 was selected from our previous experiment, in which a dose response curve for icv GnRH was established in ovx rats treated with 5 μg E_2B [69]. The dose of 50 ng elicited maximal responses in both lordosis and proceptive behaviors [69]. The number of animals in the control group was 8 (saline + GnRH), while the number of animals in the treatment group was 11 (Antide plus GnRH). The behavioral tests were conducted at 60, 120 and 240 min after GnRH administration. Previous studies both from our laboratory and others have found that lordosis behavior appears at 60 min following GnRH administration and reaches its maximal level at 120 min, declining thereafter (240 min; [54,60,74]).

2.5. Experiment 2

2.5.1. Effect of Antide on estrous behavior induced by VCS

Ovx rats were injected with 5 μg of E_2B. Forty hours later, animals were divided into two treatment groups ($n = 8$/group). One group received manual flank stimulation plus VCS and saline, and the other one received the same stimulation plus Antide. Manual flank stimulation consisted of palpations applied with the finger and thumb to both flanks and with the palm of the hand to the perineal area of the rat. VCS consisted of 150 g of pressure into the vagina and cervix through a calibrated vaginal probe [37,46] applied together with manual flank stimulation for approximately 5 s. Antide or saline (1 μl) was administered icv 1 h before stimulation. Immediately (0 min), 120 and 240 min following stimulation, females were placed in a circular plexiglas arena until they received 10 mounts with pelvic thrusts from experienced males.

2.6. Experiment 3

2.6.1. Effect of Antide on estrous behavior induced by P and MA

In this experiment we used two progestins: P, which, in the brain, is reduced at C5 (ring A-reduction) to yield initially 5α-DHP and subsequently pregnanolones by a further reduction at C3, and MA, which can not be reduced in ring A due to the presence of a double bond at C6 [60,61,79]. Ovx females were primed with 2 μg of E_2B and 39 h later, 1 μg of Antide was administered icv in 1 μl of saline. The dose of E_2B (2 μg) has been found in previous experiments to effectively prime ovx rats to the action of both P and its ring A-reduced metabolites at the dosages employed in this study. One hour later either P or MA was administered icv at a dose of 130 ng dissolved in 1 μl of oil. This dose of P has been observed in a previous study to elicit a maximal response when icv administered to ovx, E_2B-(2 μg) primed rats. Some animals ($n = 8$/group) were assigned to receive P or MA plus saline, and other rats ($n = 8$/group) received these progestins in combination with Antide. As a further control, eight females only received Antide plus the progestin vehicle (oil). Behavioral tests were carried out at 30, 120 and 240 min after progestin administration.

2.7. Experiment 4

2.7.1. Effect of Antide on estrous behavior induced by ring A-reduced metabolites of P

Animals were primed with 2 μg of E_2B for 39 h as in Experiment 3. One hour after the icv administration of 1 μg of Antide to E_2B-primed females,