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Research report

Differential effects of progesterone and genital stimulation on sequential inhibition of estrous behavior and progesterone receptor expression in the rat brain

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ABSTRACT

The effect of genital stimulation, either by vaginocervical stimulation (VCS) using a calibrated vaginal probe combined with manual flank stimulation (FS), or by mounts performed by the male, on the hypothalamus and preoptic area concentration of the progesterone receptors A (PR-A) and B (PR-B) was assessed in ovariectomized (ovx) estrogen-primed rats. VCS/FS or stimulation provided by male mounts, even without intromission, significantly decreased PR-B concentration in the hypoythalamus. Down regulation of PR produced by genital stimulation was quantitatively similar to that elicited by progesterone (P) administration. Bilateral or unilateral transection of the pelvic or the pudendal nerves prevented down regulation elicited by VCS/FS. Repeated VCS/FS elicited lordosis behavior in most ovx estrogen primed rats, but the lordosis intensity was lower than that observed in response to P. P administered to ovx estrogen primed rats, induced sequential inhibition, i.e., failure to display estrous behavior in response to a second P injection (24 h after the initial P injection). VCS/FS failed to elicit sequential inhibition, since rats responded with normal estrous behavior to the second injection of P. This suggests that down regulation by VCS, by contrast with P, failed to inhibit the subpopulation of PR involved in the facilitation of estrous behavior by P.

1. Introduction

Genital stimulation during copulation in the female rat modulates several reproductive functions including ovulation [9,26,39,47] parturition [11] and pregnancy [12,13,23,30]. Moreover, artificial vagino-cervical stimulation (VCS) activates spinal cord and brain reflexes that elicit both an immediate lordosis response and a several-hours-duration facilitation of estrous behavior [18,24,25,38,41]. The immediate lordosis induced by VCS most likely involves spinal reflex arcs that do not require hormonal stimulation, since this response can be induced in ovariectomized (ovx) or hypophysectomized [8,40] hormonally untreated rats, although estrogen priming augments the response. This immediate lordosis is not blocked by either antiestrogen or antiprogestins [1,16]. By contrast, the gradual and long lasting facilitation of estrous behavior exerted by a brief VCS (around 5 s) is blocked by the administration of antiprogestins, pointing to a role of the progesterone receptor (PR) in this effect [16].

The activity of the brain PR is known to be modulated by either progesterone (P), its normal ligand, or ligand-independent processes involving several signaling mechanisms (cAMP, cGMP, MAPK, etc.) [2,7,14,31,32,43,46] regulated by various neurotransmitters and neuromodulators. Auger et al. [1] found a rapid decrease in PR immunoreactivity in the preoptic area one hour following artificial VCS which they attributed to release of P by the adrenal glands though they could not rule out the possibility of a rapid down regulation of the PR. PR down regulation by P is associated with PR phosphorylation, a process targeting these proteins for degradation by a ubiquitin-proteasome 26S pathway [6,17,29,42]. Regulation of PR density has an important impact on the activity of a P-dependent target tissue. Thus, sequential inhibition, i.e., failure of P injection to elicit estrous behavior 24 h after an initial effective dose of P to estrogen-primed ovx rats, is related to a decrease in hypothalamic PRs [34]. It has been shown that repeated VCS produced by repeated male penile intromission, decreases both the

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intensity as well as the probability of subsequent lordosis in ovx rats treated with estradiol benzoate (E_2B) and P [22]. On the other hand, mounts without intromission as when prevented by a vaginal mask had no such inhibitory effect [22]. Since down regulation of the PR results in a decrease in estrous behavior it appears likely that the inhibitory effect of VCS is mediated by this process, i.e., down regulation of PR.

In the present study we initially investigated the capacity of artificial VCS combined with manual flank stimulation (FS) to induce down regulation of both PR isoforms, in the preoptic area and hypothalamus. In order to differentiate between the participation of pudendal and pelvic nerves in the response to VCS/FS (which activates both nerves) we compared the effect of male mounts without intromission with that in which intromission occurred on PR isoforms concentration in preoptic area and hypothalamus. In a second experiment, we determined the effect of transecting the pudendal or pelvic nerves (bilaterally or unilaterally) on the down regulation of both A and B isoforms produced by VCS/FS. Finally, we assessed in estrogen primed ovx rats the effect of VCS/FS or P on the behavioral response (lordosis and proceptive behaviors) of an injection of P given 24 h after the above mentioned procedures, i.e., sequential inhibition.

2. Materials and methods

2.1. Animals

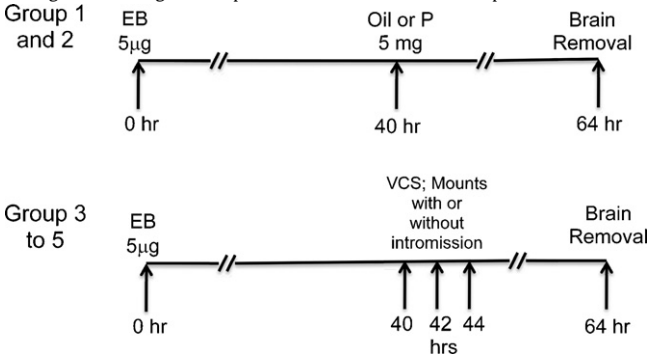
A total of sixty Sprague-Dawley female rats (240–280 g body weight), bred in our colony in Tlaxcala, were used. They were maintained under controlled temperature (23 ± 2 °C) and LD 14:10 light schedule, lights on at 20:00 h and fed rodent laboratory chow (Purina rat chow, PMI, Minnetonka, Mn, USA), and water *ad libitum*. Animal care and all the experimental procedures were performed under the Mexican Law for the Protection of Animals. Females were bilaterally ovx under ether anesthesia and housed collectively (four per cage). One week after surgery, all sixty females received a single s.c. injection of 5 μg E_2B (Sigma–Aldrich, St Louis, MO, USA) dissolved in 0.1 ml corn oil.

2.2. Experiment 1

2.2.1. Treatment groups

Rats received the following treatments: group 1, control females ($n = 8$) were injected with 5 μg E_2B and 0.1 ml/corn oil 40 h after estrogen; group 2, P (5 mg in 0.1 ml/corn oil) was administered forty hours after E_2B ($n = 4$); group 3, artificial VCS was produced by the application of a 150 g force against the vaginal cervix by a calibrated probe combined with FS ($n = 4$), the females received 10 applications of VCS at 40, 42 and 44 h after E_2B injection, i.e., a total of 30 artificial VCS; group 4, perigenital skin stimulation resulting from mounting by males which were prevented from intromitting by covering the vaginal orifice with a piece of tape ($n = 4$), this procedure was applied at 40, 42 and 44 h after E_2B injection; group 5, forty hours after E_2B injection animals from this group ($n = 4$) received a single brief VCS (5 s) to facilitate lordosis behavior and immediately placed with a vigorous male until receiving 10 intromissions. This procedure was repeated at 42 and 44 h after E_2B injection.

Diagram showing the temporal course of treatments in experiment 1.



2.3. Experiment 2

In order to assess the participation of the pelvic and pudendal nerves in the effect produced by artificial VCS, pelvic and pudendal neurectomies, both unilateral and bilateral, were performed one week after ovx and 96 h before E_2B injection.

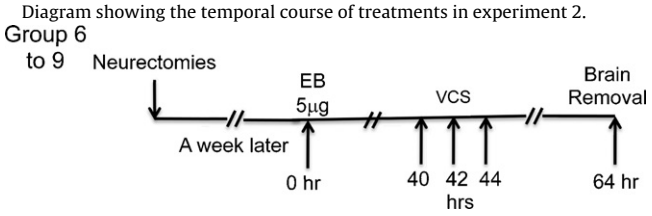
2.3.1. Surgical procedures

Surgeries were performed under general anesthesia with a mixture of Xylocine–Ketamine (15:80 mg/kg body weight). A ventral abdominal incision was performed to locate the bifurcation of the common iliac veins using gently retraction of the surrounding structures. The pelvic nerve lies perpendicular and superficial to the internal iliac vein; the more highly myelinated pudendal nerve lies parallel to the pelvic and deeper. At this level, pelvic and pudendal nerves were unilaterally and bilaterally transected. Transection removed a 2–4 mm portion of each nerve. Because pelvic nerve transection interferes with micturition, the urinary bladder was manually squeezed four times daily after surgery. Postmortem examination revealed no evidence of nerve regeneration.

2.3.2. Treatment groups

Group 6, E_2B treated rats with bilateral pelvic neurectomy ($n = 4$) subjected to artificial VCS/FS (same schedule used in group 3 from experiment 1) at 40, 42 and 44 h after E_2B injection; group 7, E_2B treated rats with unilateral pelvic neurectomy ($n = 4$) subjected to artificial VCS/FS (same schedule used in group 3 from experiment 1) at 40, 42 and 44 h after E_2B injection; group 8, E_2B treated rats with bilateral pudendal neurectomy ($n = 4$) subjected to artificial VCS/FS (same schedule used in group 3 from experiment 1) at 40, 42 and 44 h after E_2B injection; and group 9, E_2B treated rats with unilateral pudendal neurectomy ($n = 4$) subjected to artificial VCS(same schedule used in group 3 from experiment 1) at 40, 42 and 44 h after E_2B injection.

Diagram showing the temporal course of treatments in experiment 2.



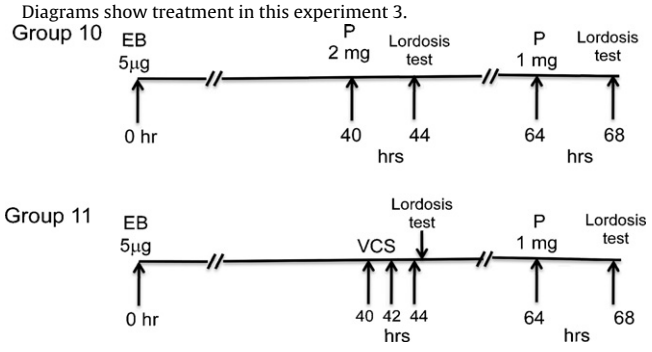
2.4. Experiment 3

This experiment explored the capacity of VCS/FS to induce sequential inhibition, i.e., a decrease in responsiveness to P following an initial exposure to P at 24 h. Twenty ovx E_2B treated rats were used in this experiment.

2.4.1. Treatment groups

Group 10, ten females received a P injection (2 mg) 40 h after E_2B; and group 11, ten females received VCS/FS as in the previous experiments at 40, 42 and 44 h following E_2B. Behavioral testing of the rats was made 4 h after P injection and immediately following the last VCS/FS (44 h). All females (groups 10 and 11) received 1 mg P 24 h later, and 4 h after the injection, they were tested for lordosis and proceptive behavior.

Diagrams show treatment in this experiment 3.



2.4.2. Behavioral testing for experiment 3

Female rats were placed in a circular arena until they received 10 mounts with pelvic thrusting from an experienced male. The lordosis quotient [LQ = (number of lordosis/10 mounts) × 100] was used to assess receptive behavior. The intensity of lordosis was quantified according to the lordosis score (LS) proposed by Hardy and De Bold [22].

This scale ranged from 0 to 3 for each individual response and, consequently, from 0 to 30 for each female that received ten mounts from the male. Proceptivity was analyzed by determining the incidence of hopping, darting, and ear wiggling during the receptivity test. The proportion of animals displaying any of these behavior patterns was used as a measure of proceptivity.

2.5. Protein extraction and Western blotting

Twenty four hours after testing (experiments 1 and 2), the females were rapidly decapitated using a guillotine, the brains were removed and the hypothalamus and preoptic area, area were excised according to the Atlas of Paxinos and Watson [37]. These regions were immediately processed for protein extraction. The hypothala-