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Differential effects of dopamine antagonists infused to the medial preoptic area on the sexual behavior of female rats primed with estrogen and progesterone

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ABSTRACT

Dopamine (DA) in the medial preoptic area (mPOA) is important for the control of appetitive aspects of sexual behavior in the female rat. Recently, following infusions of DA agonists to the mPOA of females primed with estradiol benzoate (EB) alone, we found that the ratio of D1R/D2R activity within the mPOA determines the expression of appetitive behaviors (Graham and Pfaus, 2010). To further the knowledge of this mechanism, the present experiments examined the effects of intra-mPOA infusions of selective DA receptor antagonists. Ovariectomized, sexually-experienced rats primed with EB and progesterone (P) were implanted bilaterally with cannulae aimed at the mPOA and infused with 4 doses (0, 0.25, 1.0 and 4.0 μg) of the non-selective D1R/D2R antagonist flupenthixol (FLU), and selective D1R or D2R antagonists, SCH 23390 (SCH) or raclopride (RAC), respectively, in a randomized order prior to tests of sexual behavior in bilevel chambers. The high dose of FLU significantly decreased solicitations, hops and darts, and pacing behavior. The high dose of SCH also significantly decreased solicitations. In contrast, the high dose of RAC produced an increase in pacing, and a trend toward an increase in solicitations but no other effect on sexual behavior. These results reinforce the idea that the ratio of D1R/D2R activity within the mPOA of female rats is critical for the expression of appetitive behaviors, and further that this ratio is altered by P which shifts the DA effect to a predominantly facilitative D1R activation.

1. Introduction

The medial preoptic area of the anterior hypothalamus (mPOA) is critical for the display of solicitation and other appetitive sexual behaviors in the female rat (e.g., Graham and Pfaus, 2010; Guarraci et al., 2004; Hoshina et al., 1994; Whitney, 1986), and dopamine (DA) transmission in this region appears to be of particular importance in controlling appetitive sexual behaviors. In ovariectomized (OVX) females that are primed partially with estradiol benzoate (EB-alone), administration of the non-specific DA receptor (DAR) agonist apomorphine increased appetitive behaviors while having no effect on consummatory behaviors such as lordosis. However, activation of specific DAR subtypes produces inconsistent effects: DA D1 receptor (D1R) activation decreases partial solicitations (hops and darts), whereas DA D2 receptor (D2R) activation increases both partial and full solicitations (Graham and Pfaus, 2010), but neither produce significant effects on lordosis. However, if females are primed with very high levels of estrone to make them fully receptive sexually, DAR blockade caused by the infusions of the nonspecific DA receptor antagonists flupenthixol (FLU) or haloperidol (HAL) to the mPOA results in a decreased lordosis response (Foreman and Moss, 1979).

The present experiments examined the effects of specific DAR antagonists infused bilaterally into the mPOA on appetitive and consummatory aspects of female sexual behavior. Because DAR subtypes were found to have opposite roles in partially primed females, both nonspecific and selective D1R and D2R antagonists (SCH 23310 (SCH) for D1R and raclopride (RAC) for D2R) were tested. We expected that DAR antagonists would have the opposite effects to those observed in our previous experiments with DAR agonists (Graham and Pfaus, 2010). Specifically, we hypothesized that infusions of FLU would decrease solicitations and other appetitive behaviors, and the two specific DAR antagonists would have opposing effects, such that the D1R antagonist SCH would increase appetitive behaviors as a result of shifting the ratio of D1R to D2R activation in the D2R direction, and that the D2R antagonist RAC would decrease appetitive behaviors by shifting the D1R to D2R activation ratio towards the D1R direction. We anticipated the possibility that lordosis behavior might be altered given that FLU or HAL infusions to the mPOA decrease lordosis (Foreman and Moss, 1979). However, increases in lordosis have been reported following electrolytic lesions of the mPOA (Law and Meagher, 1958; Powers and Valenstein, 1972), an effect that appears to depend on the ability of females to pace copulatory contact (Whitney, 1986). Indeed, systemic administration of HAL also increases the duration of lordosis in OVX rats

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primed with EB and P (Ismail et al., 2010), an effect that could have a dose-dependent basis in the mPOA.

2. Materials and methods

2.1. Subjects

Female Long-Evans rats, weighing 150–200 g, and male Long-Evans rats, weighing 200–250 g, were obtained from Charles River Canada, Inc. (St-Constant, QC) at six weeks of age. Pair-housed females were kept in Plexiglas cages with wood-chip bedding until cannulation, after which time they were maintained individually in the same cage. Male rats were housed with wood-chip bedding in large Plexiglas cages, four to a cage. All rats were kept in the same colony room with the room temperature kept constant at 21 °C, on a reversed 12-hour light/dark cycle, with lights off at 0800. Ad libitum tap water and regular rat chow was accessible. All animal procedures conformed to the guidelines of the Canadian Council for Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

2.2. Surgery

2.2.1. Ovariectomy

Bilaterally ovariectomies were performed on all females via a lumbar incision so that impregnation was impossible. This also allowed for hormone levels to be controlled throughout testing. General anesthesia was induced with ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (20 mg/ml), mixed in a 4:3 ratio, respectively, and administered intraperitoneally (ip) at a dose of 1 ml/kg. One week of recovery was provided prior to the onset of behavioral testing.

2.2.2. Cannula implantation

Following sexual experience training, females were bilaterally cannulated into the mPOA. Sodium pentobarbital (60 mg/ml) was used as the general anesthetic, administered ip in a volume of 1 ml/kg. Rats were then implanted with a 22-gauge stainless steel bilateral guide cannula aimed 1 mm above the mPOA (AP −0.6, ML ±0.5, DV −7.0 mm from bregma, incision bar set at 0; Paxinos and Watson, 1998), with 28-gauge cannula blockers in place, cut 0.5 mm below the cannulae. Infusion cannulae, also 28-gauge, were cut 1 mm longer than the guide cannulae. All cannula equipment was obtained from Plastics One (Roanoke, VA). A one week recovery period was then provided to the females before any infusions or testing took place.

2.3. Hormone and drug administrations

Before all sexual training and experimental trials, females were fully primed via subcutaneous injections of EB (10 μg/0.1 ml of sesame oil) and P (500 μg/0.1 ml of sesame oil) 48 h and 4 h prior to copulation, respectively.

Doses of each drug infused into the mPOA were: Flupenthixol (n = 16): High: 20.0 μg; Medium 2.0 μg; Low: 0.2 μg; SCH 23390 (n = 12): High: 4.0 μg; Medium: 1.0 μg; Low 0.25 μg; Raclopride (n = 8): High 4.0 μg; Medium: 1.0 μg; and Low 0.25 μg. All drugs were purchased from Sigma Chemical Co. (St. Louis, MO). Infusions were performed using an infusion pump (Harvard Apparatus, Pump 22) at a rate of 0.5 μl/min per side for 1 min. This gave a total volume of 1 μl. Infusion cannulae were left in place 1 min after the infusion was complete, to allow for absorption. Testing proceeded immediately after infusions. Physiological saline was used as the vehicle for each experimental drug.

2.4. Behavioral training

Females were given four 30-minute sessions of sexual experience paired with a sexually vigorous male in a bilevel chamber (Pfaus et al., 1999) before undergoing experimental testing. Sessions took place at 4-day intervals to approximate the normal ovulatory cycle of the female. Females were implanted with guide cannulae after the 4th training experience, and then given one week of surgical recovery before drug testing proceeded.

Drug tests were also administered in 4-day intervals to fully-primed females. As in our previous experiments (Graham and Pfaus, 2010), trials were randomized in a Latin squares design so that each rat completed a copulatory test following receipt of a low, medium, and high dose of one type of drug, in addition to a vehicle trial. Similar to the training sessions, females were placed in a bilevel chamber with a sexual vigorous male for a 30-minute test immediately following the drug infusion.

Experimental tests were captured onto DVD via a camcorder, and frequencies of female and male behaviors were later scored using a computerized event recorder (Cabilio, 1996). Female behaviors consisted of solicitations (characterized by a head-wise orientation to the male, followed by a 180° turn and runaway), hops and/or darts (characterized by either a hopping motion, or a burst of speed away from the male and sudden stop, without head-wise orientation; these could occur with or without each other), defensive behaviors (characterized by kicks, sideways takedowns, boxing postures, and prone positions in response to the male, as described by Barnett (1967)), level changes (going from one level of the chamber to the other), and magnitude of reflexive lordosis posture (ranging from 0, no lordosis posture, to 3, full lordosis posture, as in Hardy and Debold, 1971). Male behaviors were also scored, including the number of mounts, intromissions, and ejaculations.

2.5. Perfusions and histology

Upon completion of the fourth experimental test, females were anesthetized with sodium pentobarbital (120 mg/kg, ip) and perfused using 4% paraformaldehyde and PBS solutions. Extracted brains were placed in the 4% paraformaldehyde solution overnight, and then transferred to a 30% sucrose solution where they stayed until slicing. Slices were done coronally at 30 μm, and cannula placements were confirmed and marked in an atlas by a blind, third-party researcher.

The criterion for exclusion from statistical analyses was set so that rats with both injector cannulae ending outside the boundaries of the mPOA were exempt from the study. The end result of this was that only animals that had correct unilateral or bilateral cannulations to the mPOA were included in the analyses. Cannula placement data from subjects included in the statistical analyses are shown in Fig. 1.

2.6. Statistical analyses

A one-sample repeated measures analyses of variance (ANOVA) was performed on all sexual behaviors, both female and male, for each drug independently, as the homogeneity assumption was met because EB + P primed females show a full display of sexual behaviors. Analyses of behaviors included solicitations, hops and/or darts (H/D), defensive behaviors, lordosis quotient (LQ; the number of lordosis postures taken divided by the number of mounts, intromissions, and ejaculations), lordosis magnitudes 0–3, pacing (as indicated by level changes), mounts, intromissions, and ejaculations. Every behavior analyzed had four levels examined: the vehicle trial and the three drug infusion doses. For each significant ANOVA, post-hoc comparisons of the individual means were made using Turkey's Least Significant Difference test.