Pharmacology, Biochemistry and Behavior 94 (2010) 482–487

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Acute cannabinoid administration attenuates female socio-sexual motivation

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ARTICLE INFO

Article history:
Received 13 August 2009
Received in revised form 5 November 2009
Accepted 12 November 2009
Available online 17 November 2009

Keywords:
Sexual motivation
Social
Estrous
Cannabinoid
CP55,940
Estrogen
Progesterone

ABSTRACT

Endocannabinoids may normally inhibit the generation and expression of female estrous behaviors. Previous work in our laboratory demonstrated that acute administration of a CB_1 receptor antagonist (AM251) increased sexual incentive motivation in estrous female rats. The current experiment examined the effect of CP55,940, a synthetic cannabinoid agonist, on sexual motivation. Seventy-two ovariectomized female Long–Evans rats were tested for their socio-sexual motivation via a runway methodology. Baseline motivation to approach and maintain close proximity to an empty goalbox, a female conspecific, and a male conspecific was assessed over six trials. Subjects were then grouped into nine experimental conditions and re-tested for their socio-sexual motivation after one of three possible hormonal treatments and three drug doses. Hormone treatments were: oil (nonestrous), 10 μg estradiol benzoate (partially estrous), and 10 μg estradiol + 500 μg progesterone (fully estrous). Drug doses were: 0, 20, or 40 μg/kg CP55,940 (IP, 30 min prior to testing). As expected, hormonal priming with both estradiol and progesterone significantly increased sexual motivation in females that did not receive drug treatment. This occurred even though females were kept sexually-naïve throughout the experiment. CP55,940 dose-dependently attenuated sexual motivation for a male target in estrous females; the 40 μg/kg dose completely blocked sexual motivation. However, this same dose also significantly reduced social motivation for another female. Cannabinoid agonists reduce female sexual motivation, either directly by inhibiting estrus or indirectly by increasing social anxiety.

1. Introduction

There is significant evidence that both exogenous and endogenous cannabinoids can alter aspects of male and female copulatory behavior (reviewed in Gorzalka and Hill, 2006). In male rats, cannabinoid agonists tend to inhibit both appetitive and consummatory variables. Cannabinoids, including Δ^9-tetrahydrocannabinol (THC), increase mount, intromission, and ejaculation latencies and reduce intromission frequency (Ferrari et al., 2000; Gorzalka et al., 2008; Martinez-Gonzalez et al., 2004; Murphy et al., 1994). In contrast, cannabinoid antagonists can induce erections, reduce the number of intromissions necessary for ejaculation, and reduce ejaculation latencies (Castelli et al., 2007; Gorzalka et al., 2008; Melis et al., 2004, 2006; Succu et al., 2006). Cannabinoid effects on female sexual behavior tend to be less consistent. Several laboratories have shown that cannabinoid agonists can enhance receptivity in females primed with estrogen (Gordon et al., 1978; Mani et al., 2001; Turley and Floody, 1981). Mani et al. (2001) have suggested that cannabinoid-induced dopamine release may subsequently activate progesterone receptors to induce full behavioral estrus. However, under some experimental conditions and at higher doses, cannabinoid agonists significantly interfere with female sexual behaviors (Ferrari et al., 2000; Gordon et al., 1978).

The majority of work conducted on cannabinoid regulation of female sexual behavior has focused on receptivity. While the lordosis reflex is a convenient and readily measurable behavior, it is perhaps not the most externally valid choice if one's scientific goal is to model women's sexuality. Women do not experience overt changes in receptivity across the menstrual cycle and are capable of engaging in sexual intercourse at any time (Thornhill and Gangestad, 2008). However, in rats, non-human primates, and women, sexual motivation and mate preference are regulated by cyclic fluctuations in steroid hormones (Beach, 1976; Regan, 1999; Wallen, 2001). Precopulatory motivation is best assessed through methodologies that completely dissociate appetitive variables from performance (López et al., 1999), as in approach behavior tests where the subject and target incentive are prevented from physically interacting (Agmo et al., 2004). Our laboratory uses a simple, unconditioned approach methodology to assess socio-sexual motivation in both male and female rats. Subjects traverse a straight-arm runway to approach a goalbox containing a motivationally-relevant target, such as an opposite-sex conspecific. Attraction to the goalbox target is assessed by recording the amount of time that the subject spends in close proximity.

We have previously reported that AM251, a cannabinoid antagonist/reverse agonist, increases sexual motivation in estrous females tested within the runway apparatus (López et al., 2009). Pre-

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doi:10.1016/j.pbb.2009.11.002

treatment with either 2 or 4 mg/kg of AM251 significantly increased the amount of time that female subjects spent near male, but not female, targets. Interestingly, this effect was only noted in females who were hormonally-primed with both estradiol and progesterone prior to behavioral testing. AM251 treatment also significantly increased the number of proceptive displays emitted by estrous females in a brief, non-paced mating test. Based on this work, we suggested that endocannabinoids may play a tonic, inhibitory role in the regulation of female estrous behavior.

The current experiment was designed to specifically assess the effect of a cannabinoid CB_1 receptor agonist, CP55,940, on female sexual motivation, across a variety of hormonal conditions. This work is a direct extension of our previous experimentation with AM251 (López et al., 2009). Based on that work, we hypothesized that CP55,940 would dose-dependently attenuate sexual motivation in estrous females.

2. Method

2.1. Subjects

A total of 76 female and 4 male Long–Evans rats (Charles River Laboratories, Wilmington, MA) were used. Female subjects were ovariectomized (OVX) at Charles River Laboratories 1 week prior to arrival at our vivarium and were given a minimum of 2 weeks post-surgery recovery time before being subjected to any experimental procedures. Subjects were approximately 70 days old at the start of behavioral testing. All females were pair-housed in plastic cages with woodchip bedding; males were individually housed in identical cages in the same room. Food and water were provided *ad libitum*. The vivarium environment was humidity and temperature controlled (~22 °C), and subjects were maintained under a reverse 12:12 light–dark schedule (lights on 2200 h–1000 h). All animals were handled daily by the experimenters for 1 week prior to the behavioral testing. The care and use of animals, and all aspects of the experimental protocol, were reviewed and approved by the campus Institutional Animal Care and Use Committee (IACUC) for compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Hormones and drug

Steroid hormones were purchased from Sigma-Aldrich (St. Louis, MO). Estradiol benzoate (EB) was prepared in a sesame oil vehicle, and progesterone (P) was prepared in propylene glycol. Both hormones were injected subcutaneously at a volume of 0.1 ml. CP55,940 (Tocris Biosciences, Ellisville, MO) was prepared as in Braida et al. (2004), in a solution of cremophor, ethanol, and saline (1:1:18). Stock solution was gently sonicated before being aliquotted into three separate vials. Vials were stored at −10 °C until use, which occurred within 10 days of solution preparation.

CP55,940 was administered intraperitoneally (IP), in a volume of ~1 ml/kg, 30 min before the behavioral testing, as in previous research with this compound (e.g. Genn et al., 2004). Two doses were used: 20 μg/kg and 40 μg/kg. The lower dose was primarily chosen because it is rewarding to rats and therefore may model a human "recreational" dose (Braida et al., 2001). Moreover, 20 μg/kg does not significantly affect locomotion (Genn et al., 2004; Kosiorek et al., 2003) or increase anxiety in a social interaction test (Genn et al., 2004). After our initial trials indicated that 20 μg/kg was having a modest but non-significant effect on sexual motivation, we extended our dose analysis to include 40 μg/kg. This higher dose has been shown to decrease locomotor activity and increase anxiety under some experimental conditions (Genn et al., 2004; Kosiorek et al., 2003).

2.3. Runway apparatus

Motivational testing occurred in two identical straight-arm runways, each consisting of a wooden startbox (20×20×30 cm), a wooden alley (160×10×15 cm), and a cylindrical Plexiglas goalbox (50 cm diameter×30 cm height). Plexiglas guillotine doors separated the startbox from the alley, and the alley from the goalbox. A removable Plexiglas partition divided the goalbox arena into two semicircular halves. Thirty-five holes (1 cm diameter) drilled into the partition provided airflow between the halves. This partition allowed subjects to perceive visual, olfactory, and scent cues from the target animal, while preventing direct physical contact. Fig. 1 depicts a schematic representation of the runway apparatus.

Three infrared photocell emitter-detector sensor pairs built into each runway detected subject motion. Sensor #1 was placed 15 cm deep within the goalbox and was only triggered when the subject's entire head and body entered the goalbox. Sensor #2 was placed within the alley, 25 cm away from the goalbox door, and only became active after sensor #1 was triggered. Sensor #3 was located just outside the startbox. Sensors #1 and #2 allowed for measurement of a subject's proximity time (PT). An electronic timer started when the subject first entered the goalbox and triggered sensor #1. If the subject's entire body left the goalbox and triggered sensor #2, the timer stopped. If the subject re-entered the goalbox and triggered sensor #1, the timer would start again. This continued for a period of three minutes following the initial entry of the subject into the goalbox.

In addition to PT, we also counted subject "retreats." We defined a retreat as a complete return to the startbox after the subject had entered the goalbox. Every time the subject made a circuit between sensor #1 (goalbox) and sensor #3 (startbox), an electronic counter increased by one. Previous research has indicated that retreats can be a reflection of subject ambivalence over goalbox events and a behavioral manifestation of approach–avoidance conflict (Ettenberg, 2004, 2009). However, we primarily used retreats as a simple, nonspecific measure of subject ambulation within the apparatus, so that we could assess drug effects on locomotor capacity.

In numerous prior experiments, we have successfully used this apparatus and methodology to assess socio-sexual motivation in both male (López and Ettenberg, 2001, 2000, 2002; López et al., 1999) and female rats (López et al., 2009, 2007; Nofrey et al., 2008).

2.4. Procedure

2.4.1. Habituation and baseline phase

All runway testing took place under red-light illumination between 13:00 and 18:00 h (the middle of the subjects' active phase). It should be noted that neither subjects nor targets possessed any sexual experience prior to runway testing. This is in contrast to our most recent work on female sexual motivation (López et al., 2009). Subjects were given three habituation sessions (10 min each) within an empty runway on consecutive days, to familiarize them with the apparatus.

Baseline socio-sexual motivation of 72 female subjects was measured over the next 6 days. Each subject was tested in a nonestrous state for their motivation to maintain close proximity to one of three different goalbox targets: an adult male, an OVX (nonestrous) female, or an empty goalbox. Subjects did not receive hormone or drug treatment. Subjects ran one trial per day, and all ran for the same target on any given day. Thus, two trials per goalbox target were conducted during baseline; scores across these two trials were averaged. The day-to-day order of targets presented during baseline was randomly determined. Four OVX females and 4 intact males were used as targets.

Prior to beginning a day's trials, the assigned target (if a female or male conspecific) was confined to the goalbox for a period of 10 min to infuse the area with scent cues. The partition was then introduced