Behavioural Brain Research 271 (2014) 39–42

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short Communication

Estradiol facilitation of cocaine-induced locomotor sensitization in female rats requires activation of mGluR5

Luis A. Martinez[a,*], Brittni M. Peterson[a,b], Robert L. Meisel[a,b], Paul G. Mermelstein[a,b]

[a] Department of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA
[b] Graduate Program of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA

HIGHLIGHTS

- mGluR5 blockade disrupted estradiol-enhancement of locomotor sensitization.
- There was no effect on non-ambulatory locomotor responses (e.g., rearing).
- Physiological responses to estradiol were also not disrupted by mGluR5 blockade.

ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 20 May 2014
Accepted 24 May 2014
Available online 2 June 2014

Keywords:
Nucleus accumbens
Estrogen
Drug addiction
Behavioral sensitization

ABSTRACT

In comparison to men, women exhibit enhanced responsiveness to the stimulating and addictive properties of cocaine. A growing body of evidence implicates the steroid hormone estradiol in mediating this sex difference, yet the mechanisms underlying estradiol enhancement of behavioral responses to cocaine in females are not known. Recently, we have found that estrogen receptor alpha (ERα) functionally couples with the metabotropic glutamate receptor 5 (mGluR5) to mediate the effects of estradiol on both cellular activation as well as dendritic spine plasticity in brain regions involved in cocaine-induced behavioral sensitization. Thus, we sought to determine whether mGluR5 activation is required for the facilitative effects of estradiol on locomotor responses to cocaine. To test this hypothesis, ovariectomized (OVX) female rats were tested for locomotor activity on the first and fifth days of daily systemic injections of cocaine. For the 2 days prior to each locomotor test, animals were injected with the mGluR5 antagonist MPEP (or vehicle) and estradiol (or oil). MPEP treatment blocked the facilitative effects of estradiol on cocaine-induced locomotor sensitization, without affecting acute responses to cocaine or the inhibitory actions of estradiol on weight gain. Considered together, these data indicate that mGluR5 activation is critical for the actions of estradiol on cocaine-induced behavioral sensitization.

Abuse of psychostimulants such as cocaine differs between men and women. Women begin using psychostimulants at an earlier age than men, escalate their use more quickly, and report greater subjective effects of these drugs [1]. Notably, responses to cocaine in women vary across the menstrual cycle with elevated responsiveness during the follicular phase [2], highlighting the contribution of estradiol to this differential response to cocaine. Indeed, studies in rodents have demonstrated that sex differences in the behavioral response to cocaine are eliminated following ovariectomy, and are restored following treatment with estradiol [3,4]. Despite such observations, little is known regarding the underlying neural mechanisms that mediate the facilitative effects of estradiol on cocaine-induced behaviors.

There is a growing body of evidence implicating metabotropic glutamate receptors (mGluRs) as an underlying mechanism for many of the neuroanatomical and behavioral effects of estradiol [5]. Recently, we found that estrogen receptor alpha (ERα) is functionally coupled to mGluR5 in striatal medium spiny neurons (MSNs) [6], an interaction that appears to be critical for plasticity within brain areas that regulate behavioral sensitization to cocaine. Similar to several recent studies examining the effects of cocaine [7,8], estradiol decreases dendritic spine density of MSNs in the core subdivision of the nucleus accumbens 24–48 h after hormone administration [9]. Furthermore, pretreatment with the mGluR5 antagonist MPEP blocks this effect of estradiol [10]. Given that mGluR5 signaling has previously been implicated in the locomotor responses to cocaine [11,12], we hypothesized that in

* Corresponding author. Tel.: +1 612 625 0784; fax: +1 612 626 5009.
E-mail addresses: lamartin@umn.edu, luisbm1634@gmail.com (L.A. Martinez).

http://dx.doi.org/10.1016/j.bbr.2014.05.052
0166-4328/

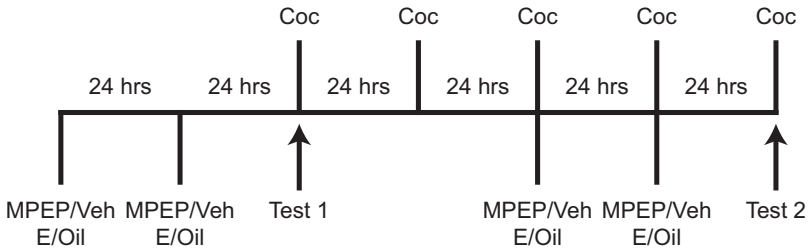

Fig. 1. Timeline of experimental manipulations. OVX female rats were injected with MPEP or vehicle (Veh), followed by estradiol (E) or oil, on days 1, 2, 5, and 6 (n = 12–13 per group). Cocaine (Coc) was injected on days 3–7. Locomotor activity was assessed on the first and fifth Coc injection days.

females ERα/mGluR5 signaling is required for estradiol facilitation of cocaine-induced locomotor sensitization.

Ovariectomized Sprague–Dawley rats were purchased from Harlan Laboratories (Madison, WI, USA) at 175–199 g, and pair housed upon arrival in polycarbonate cages with wire mesh tops. Females were maintained on a 12:12 h light:dark cycle (lights on at 6 a.m.), with all behavior testing occurring between the hours of 9 a.m. and 2 p.m. Food and water were available ad libitum. Animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (8th ed.) and approved by the University of Minnesota Institutional Animal Care and Use Committee.

Throughout the experiment, females were injected s.c. with 2 μg of estradiol (17β-estradiol; Sigma, St. Louis, MO, USA) in 0.1 ml cottonseed oil (or cottonseed oil alone), on a 2 days on, 2 days off schedule (Fig. 1). Females were also injected i.p. with 1 mg/kg/ml MPEP (2-methyl-6-(phenylethynyl)pyridine hydrochloride; Abcam, Cambridge, MA, USA) in saline (or saline vehicle alone) 30 min prior to each hormone injection. We found previously that this dose of MPEP blocks estradiol-induced changes in dendritic spines within the nucleus accumbens core when measured 24 h later, but has no effect on spine density in the absence of estradiol treatment [10]. Beginning on the third day of the experiment (the day immediately following the second day of hormone injections), females were injected i.p. for 5 consecutive days with 15 mg/kg/ml of cocaine (cocaine hydrochloride; Sigma) in saline. This pattern of cocaine injections was chosen because it induces modest (if any) sensitization in untreated OVX female rats [1,4], thereby allowing us to more clearly identify the facilitative effect of estradiol on sensitization as well as any potential blockade of that effect following MPEP treatment.

Locomotor activity was assessed in clear, polycarbonate open field chambers (47.5 cm × 25.5 cm × 20.5 cm) containing corncob bedding. Females were initially placed into a chamber for 30 min (Habituation session), injected with cocaine, and then immediately returned to that chamber for 60 min (Test session). Each chamber was situated within a sensing frame (Kinder Scientific, Poway, CA, USA) that generated an X–Y grid of photobeams within the chamber. Data from the sensing frames were transmitted to a computer running Motor Monitor software (Kinder Scientific). This software further discriminated beam breaks into ambulations and fine movements. Ambulations were defined as a change of the animal's entire body position on the X–Y grid. Fine movements were defined as all beam breaks that did not meet the criterion for an ambulation. Fine movements therefore comprised a range of behaviors, including grooming and sniffing. The number of rears (elevation of the animal onto its hind paws with fore paws placed upon the wall of the chamber) was also quantified, by a researcher blind to the experimental condition of the animal. At the conclusion of the Test session, females were returned to their home cages.

All data were analyzed using SPSS for Macintosh, version 20.0 (IBM Corp, Armonk, NY, USA). Data were first examined to determine if the assumptions of parametric statistical tests were met. For all statistical tests, results were considered to be significant if $p < .05$. Body weight was subjected to a mixed-design factorial ANOVA, with time (initial weight, weight at first cocaine test, and weight at fifth cocaine test) as a repeated factor, and drug (MPEP or vehicle) and hormone (estradiol or oil) as independent factors. Significant time × hormone interactions were further examined for the effect of time within each hormone treatment group using paired-samples t-tests (error term derived from interaction analysis), with Bonferroni correction for multiple comparisons. The number of ambulations, fine movements, and rears during the first and fifth Habituation and Test sessions were also examined via factorial ANOVAs, and significant time × drug × hormone interactions were further examined for the effect of time within each treatment group using paired-samples t-tests (error term derived from interaction analysis).

MPEP injections did not significantly affect locomotor responses during Habituation sessions. Females performed fewer ambulations ($F(1,45) = 90.61$, $p = .000$), fine movements ($F(1,46) = 60.35$, $p = .000$), and rears ($F(1,43) = 45.63$, $p = .000$) in the fifth vs. the first session (data not shown). However, there were no significant effects of drug, hormone, or drug × hormone interactions.

MPEP treatment blocked the estradiol facilitation of sensitized ambulatory activity following repeated cocaine injections. The effects of estradiol treatment and test session on ambulatory activity differed across drug treatment groups, as evidenced by a significant time × drug × hormone treatment interaction ($F(1,43) = 4.49$, $p = .040$) (Fig. 2). Specifically, females treated with estradiol + vehicle had significantly higher ambulations in the fifth vs. first Test sessions ($t(11) = -2.61$, $p = .024$), an effect that was not observed in any other treatment group. There were no significant effects of drug, hormone, or significant drug × hormone interaction on the initial ambulatory responses to cocaine.

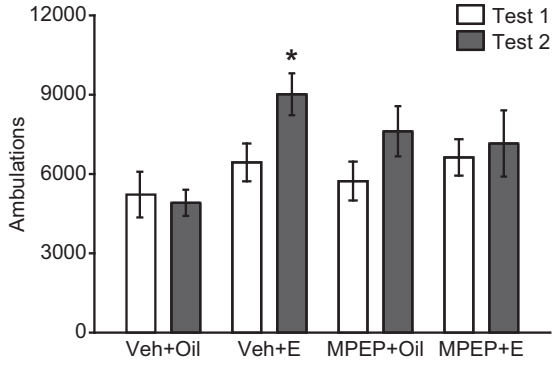

Fig. 2. Estradiol enhancement of cocaine-induced ambulations is dependent on mGluR5. In estradiol (E) treated females, ambulations (mean ± SEM) were higher in response to the fifth cocaine (Coc) vs. first Coc injection (Test 2 vs. Test 1, $^*p < .05$ (paired-samples t-test)). This hormone effect was not observed when MPEP was administered 30 min prior to estradiol. In addition, MPEP alone did not affect Coc-induced ambulations.