Psychoneuroendocrinology 63 (2016) 59–67

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

The neurosteroidogenic enzyme 5α-reductase modulates the role of D_1 dopamine receptors in rat sensorimotor gating

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ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form 21 August 2015
Accepted 14 September 2015

Keywords:
Finasteride
5α-Reductase
Dopamine
Neurosteroids
Prepulse inhibition of the startle

ABSTRACT

Neurosteroids exert diverse modulatory actions on dopamine neurotransmission and signaling. We previously documented that the enzyme 5α-reductase, which catalyzes the main rate-limiting step in neurosteroid synthesis, is required for the behavioral responses of Sprague–Dawley rats to non-selective dopaminergic agonists, such as the D_1–D_2 receptor agonist apomorphine. Specifically, systemic and intra-accumbal administrations of the 5α-reductase inhibitor finasteride countered apomorphine-induced deficits of sensorimotor gating, as measured by the prepulse inhibition (PPI) of the startle reflex; the classes of dopamine receptors involved in these effects, however, remain unknown. Prior rodent studies have revealed that the contributions of dopamine receptors to PPI regulation vary depending on the genetic background; thus, we analyzed the effect of finasteride on the PPI deficits induced by selective dopamine receptor agonists in Long–Evans (a strain exhibiting PPI deficits in response to both D_1 and D_2 receptor agonists) and Sprague–Dawley rats (which display PPI reductions following treatment with D_2, and D_3, but not D_1 receptor agonists). In Long–Evans rats, finasteride opposed the PPI deficits induced by activation of D_1, but not D_2 receptors; conversely, in Sprague–Dawley rats, finasteride prevented the reductions in %PPI and accumbal dopamine extracellular levels caused by selective stimulation of D_3, but not D_2 receptors; however, the effects on %PPI were not confirmed by analyses on absolute PPI values. Our findings suggest that 5α-reductase modulates the effects of D_1, but not D_2 receptor agonists on sensorimotor gating. These data may help elucidate the role of neurosteroids in neuropsychiatric disorders featuring PPI deficits, including schizophrenia and Tourette syndrome.

1. Introduction

The prepulse inhibition (PPI) of the acoustic startle reflex is one of the best-validated parameters to measure *sensorimotor gating*, namely the suppression of a motor response by a sensory stimulus. PPI consists in the reduction of the startle response triggered by a weak pre-stimulus immediately preceding the startle-eliciting burst (Hoffman and Ison, 1980). This endophenotype has garnered substantial interest in neuropsychiatric and behavioral research. Indeed, PPI deficits have been observed in several disorders, including schizophrenia and Tourette syndrome (Braff et al., 2001); furthermore, the accessibility of PPI as a valid testing paradigm for humans and experimental animals makes it particularly attractive for translational studies (Swerdlow et al., 1999).

Dopamine plays a key role in the orchestration of PPI. Rich evidence has shown that dopaminergic agonists produce robust PPI deficits in rats and mice (Geyer et al., 2001); the specific contribution of dopaminergic receptors to the modulation of sensorimotor gating, however, varies across different rodent models. In Sprague–Dawley (SD) albino rats, PPI deficits are elicited by agonists for dopamine D_2 and D_3, but not D_1 receptors (Peng et al., 1990; Bristow et al., 1996); nevertheless, D_1 receptor activation has been shown to be directly involved in the PPI deficits induced by non-specific dopaminergic agonists, such as apomorphine (APO) (Hoffman and Donovan, 1994). Conversely, we recently found that the selective and independent activation of D_1 and D_2 receptors produces PPI deficits in the hooded Long–Evans (LE) strain (Mosher et al., 2015).

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http://dx.doi.org/10.1016/j.psyneuen.2015.09.014
0306-4530/

We previously showed that, in SD rats, the PPI deficits induced by non-selective dopaminergic agonists are countered by inhibition of 5α-reductase (5αR) (Bortolato et al., 2008), the enzyme catalyzing the rate-limiting step in neurosteroid synthesis (i.e. the irreversible saturation of the 4,5 double bond of the A ring of Δ^4-3 ketosteroids such as pregnenolone and progesterone) (Martini et al., 1993). Accordingly, systemic and intra-accumbal injections of the selective 5αR inhibitor finasteride (FIN) attenuates the PPI deficits induced by non-selective dopaminergic agonists in SD rats (Bortolato et al., 2008). In parallel with these preclinical results, preliminary results collected by our group suggest that FIN may have elicited therapeutic properties in patients affected by chronic schizophrenia (Koethe et al., 2008) and Tourette syndrome (Bortolato et al., 2007; Muroni et al., 2011). Notably, the anti-dopaminergic effects of FIN and other 5αR blockers were not accompanied by extrapyramidal side effects. While these premises point to these agents as promising options for the therapy of these neuropsychiatric conditions, the specific involvement of dopamine receptors in the PPI-enhancing effects of FIN remains elusive. Based on this background, in the present study we investigated the specific contributions of different dopamine receptor subtypes on sensorimotor gating using SD and LE rats.

2. Material and methods

2.1. Animals

A total of 390 male SD and 102 LE rats (Harlan; Milan, Italy and Indianapolis, IN) weighing between 250 and 350 g were used for these experiments. Animals were group-housed in cages ($n = 3$–4) with *ad libitum* access to food and water. Rooms were maintained at 22 ± 0.2 °C on reversed 12-h light/dark cycle (with lights off at 07:00 PM). Each animal was used only once throughout the study and all efforts were made to minimize animal suffering. PPI and microdialysis studies occurred between 11:00 AM and 5:00 PM. Care was taken in ascertaining the uniformity of all husbandry conditions across the two facilities where the experiments were performed (University of Kansas, USA and University of Cagliari, Italy). All experimental procedures were in compliance with the National Institute of Health guidelines and approved by the Institutional Animal Use Committees of the University of Kansas and Cagliari.

2.2. Drugs

The following drugs were used in the present study: finasteride (FIN), (*R*)-(−)-apomorphine hydrochloride, SKF 82,958 hydrobromide, (−)-quinpirole hydrochloride, sumanirole maleate, (+)-PD 128,907 hydrochloride, SCH 23,390 and GR 103,691 (Sigma–Aldrich, Milan, Italy). FIN was suspended in a vehicle (VEH) solution containing 5% Tween 80 and 95% saline, while the other drugs were dissolved in saline (SAL) solution. Drug doses are based on mg/kg of salts. All solutions were freshly prepared on the day of testing and administered subcutaneously (SC) and intraperitoneally (IP) in an injection volume of 1 and 2 ml/kg body weight, respectively. The doses and the latency time of the drugs used in these experiments were determined by our previous studies and in accordance with those commonly used in PPI studies on rats (Wan et al., 1996; Geyer et al., 2001; Bortolato et al., 2008; Mosher et al., 2015).

2.3. Acoustic startle reflex and PPI

Startle and PPI testing were performed as previously described in Mosher et al. (2015). The apparatus used for detection of startle reflexes (Med Associates, St Albans, VT, USA) consisted of six standard cages placed in sound-attenuated chambers with fan ventilation. Each cage consisted of a Plexiglas cylinder of 9 cm diameter, mounted on a piezoelectric accelerometric platform connected to an analogue-digital converter. Two separate speakers conveyed background noise and acoustic bursts, each one properly placed so as to produce a variation of sound within 1 dB across the startle cage. Both speakers and startle cages were connected to a main PC, which detected and analysed all chamber variables with specific software. Before each testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates. Rats were first subjected to a *pre-test* session, during which they were exposed to a sequence of seventeen trials, consisting of 40-ms, 115-dB burst, with a 70-dB background white noise. Experimental groups were defined based on the average startle amplitude of the rats, so as to maintain comparable values of average startle response across all groups.

Three days after the pre-test session, rats were treated and underwent a *test session*. This session featured a 5-min acclimatization period, with a 70-dB background white noise, which continued for the remainder of the session. The acclimatization period was followed by three *blocks*, each consisting of a sequence of trials: the first and the third block consisted of five pulse-alone trials of 115 dB (identical to those used in the pre-test session). The second block consisted of a pseudorandom sequence of 50 trials, including 12 pulse-alone trials, 30 trials of pulse preceded by 74, 78, or 82 dB pre-pulses (10 for each level of pre-pulse loudness), and 8 no-pulse trials, where only the background noise was delivered. *Inter-trial intervals* (i.e, the time between two consecutive trials) were selected randomly between 10 and 15 s.

The %PPI was calculated only on the values relative to the second period, as well, using the following formula:

$$100 - \left(\frac{\text{mean startle amplitude for prepulse pulse trials}}{\text{mean startle amplitude for pulse alone trials}}\right) \times 100$$

For both the pre-test and the test session, the *interstimulus interval* (i.e., the duration between the prepulse and the pulse in each trial) was kept at 100 ms. The selection of this interstimulus interval was based on previously published experiments from our group (Mosher et al., 2015), which showed this parameter to be optimally suited to reveal PPI deficits in response to selective dopamine receptor agonists in LE and SD rats.

A major caveat in %PPI computation is that increases or reductions in startle magnitude can respectively lead to artifacts, due to "ceiling" or "floor" effects (Swerdlow et al., 2000).

In consideration of FIN's ability to reduce startle magnitude (Bortolato et al., 2008), whenever FIN was found to produce significant effects on both startle magnitude and %PPI, we performed confirmatory analyses of ΔPPI values. This parameter was calculated as the absolute differences between startle magnitudes on pulse-alone and prepulse + pulse trials (Bortolato et al., 2004).

2.4. Microdialysis

Microdialysis experiments were performed as previously described in Devoto et al. (2012). SD rats were deeply anesthetized with Equithesin (containing, per 100 ml, 0.97 g pentobarbital, 2.1 g $MgSO_4$, 4.25 g chloral hydrate, 42.8 ml propylene glycol, 11.5 ml 90% ethanol; 5 ml/kg, i.p.) and placed in a Kopf stereotaxic apparatus. The skull was exposed and a hole was drilled for the implant of vertical microdialysis probes (membrane AN 69-HF, Hospal-Dasco, Bologna, Italy; cut-off 40,000 Da, 2 mm active membrane length), in the nucleus accumbens shell [AP +1.7, L ±0.8, V −7.8 from the bregma, according to the coordinates of Paxinos and Watson (1997)]. The probes were secured to the scull by means of two screw and cranioplastic cement. The day after probe implantation, artificial cerebrospinal fluid (147 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$,