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Progesterone reduces hyperactivity of female and male dopamine transporter knockout mice

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Abstract

There are gender differences in prevalence, course, and/or prognosis of schizophrenia. Yet, neurobiological factors that may account for the more favorable outcomes of women with schizophrenia are not well understood. Evidence that the steroid hormone, progesterone (P₄), may influence mood and/or arousal among some people with schizophrenia led us to examine the effects of P₄ on dopamine transporter knockout (DATKO) mice, an animal model of schizophrenia. Our hypothesis was that P₄ would have greater effects than vehicle to improve the behavioral phenotype of DATKO, more so than wildtype, mice. Young adult, male and female DATKO mice and their wildtype counterparts were subcutaneously administered P₄ (10 mg/kg) or vehicle 1 h prior to testing in pre-pulse inhibition (PPI), activity monitor, or open field. DATKO mice had impaired PPI compared to their wildtype counterparts, but there was no effect of P₄. In the activity monitor, DATKO mice showed significantly greater distance traveled during the 60 min test compared to wildtype controls. In the open field, DATKO mice made a significantly greater number of total, but fewer central, entries than did wildtype mice. Administration of P₄ decreased the hyperactivity of DATKO mice in the activity monitor and open field, but did not alter motor behavior of wildtype mice. P₄ increased the number of central entries made by DATKO and wildtype mice. Thus, P₄ administration to DATKO female or male mice partially attenuated their hyperactive phenotype.

Keywords

Estrogen; Hippocampus; Neurosteroid; Affect; Reproductive experience

1. Introduction

Schizophrenia is characterized by deficits in social, affective and cognitive functioning. Gender differences in schizophrenia include that some women have a lower incidence, later age of onset, fewer hospitalizations, and better response to antipsychotics than do some men [24,27,31,58]. As with other gender/sex differences that are influenced by hormonal status, there is evidence for a role of hormones to influence expression of symptoms in schizophrenia. In support, some women have a greater occurrence of negative symptoms and/or may require higher dosages of antipsychotics, when endogenous progesterone (P₄)

levels are low, during the follicular phase or post-menopause [21,24,58]. Thus, there is clinical evidence for P₄ to influence the pathophysiology of schizophrenia.

In animal models, there is evidence that P₄ can modulate the expression of some behaviors that are altered in schizophrenia. First, P₄ facilitates sexual and/or social behaviors of female rodents, in part through its actions in the Ventral Tegmental Area (VTA), which is notable for the many dopamine (DA) cell bodies located there. Some of P₄'s actions in this region may be through DA signaling. Activation and/or attenuation of P₄'s actions via DA type 1 receptors in the VTA, respectively, facilitates and inhibits, sexual responses of rodents [61]. Second, P₄ also has effects on arousal and affective measures. In support, when administered to aged mice, P₄ (10 mg/kg, subcutaneously, SC), compared to vehicle, increased: the number of central entries in the open field, open quadrant time in the elevated zero maze, time spent in the mirror-chamber, time spent in light chamber in the dark/light transition task, and increased punished drinking in the Vogel conflict task [14]. Interestingly, similar effects of P₄ to enhance sexual receptivity and arousal were observed in older C57BL/6 mice and also P₄ receptor knockout mice (PRKOs) [15]. Third, P₄ can enhance cognitive function in tasks mediated by the nucleus accumbens (conditioned place preference), cortex (object recognition; T maze), and/or hippocampus (object recognition, water maze, conditioned fear), areas which are DA sensitive and that the VTA projects to [8,10,12,65]. Moreover, P₄ can have these effects in wildtype and PRKO mice. Together, these data suggest that P₄ has effects on some normative functional process that are atypical in schizophrenia and that some of these effects may be at least/in part, independent of action at PRs.

Dopaminergic inputs from the VTA play a key role in arousal and affective function in animals. The DA and serotonin (5-HT) systems have been implicated in striatal dysfunction associated with schizophrenia and may also play a role in reward and/or susceptibility to drug abuse [4,26]. As such, a greater understanding of hormones' effects through these systems is important.

The DA transporter (DAT) is a plasma membrane transport protein that controls extracellular DA concentrations and is an important target for a variety of therapeutic agents [51,53,59,61,62]. DAT knockout mice (DATKOs) exhibit elevated interstitial levels of dopamine and a range of behavioral alterations, including poor cognitive function [40], hyperactivity, and some stereotyped and/or perseverative behavior [61,62]. DATKO mice also have impaired pre-pulse inhibition (PPI), a model of sensorimotor gating in schizophrenia [67]. To date, the effects of P₄ on behavior of DATKO mice and their wildtype counterparts have not been reported. Evidence from our laboratory suggests that P₄ may influence sexual, social, cognitive and/or affective behaviors in part through its actions on the DA systems. As such, we wished to test the hypothesis that P₄ may reduce hyperactivity of DATKO, but not wildtype, mice.

2. Methods

These methods were pre-approved by The Institutional Animal Care and Use Committees at the University at Albany, State University of New York and The Tohoku University Graduate School of Medicine (Sendai, Japan).

2.1. Subjects

DATKO mice are used as a model for schizophrenia [50,59]. Individual DATKO strains used in this report have been described previously [61,62]. Intact male and female wildtype and DATKO mice ($N=120$; $n=15$ /group) were bred and obtained from the colony at Tohoku University Graduate School of Medicine. DATKO and wild-type littermates were

obtained by heterozygote crosses that had been previously generated on 129Sv-C57BL/6J mixed genetic background [60].

2.2. Housing

Offspring were weaned at postnatal day 28 and group housed, segregated by sex, in a temperature- and light-controlled colony (lights on at 0800 h, lights off at 2000 h), with food and water available *ad libitum*.

2.3. Genotype

Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tails, as previously described [14–16].

2.4. Subcutaneous P₄-priming

All mice were randomly assigned to either P₄ (10 mg/kg) or vehicle (sesame oil) condition. P₄ was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sesame oil to a concentration of 10 mg/ml. Mice received SC P₄ or vehicle injections 1 h prior to behavioral testing. This P₄ regimen was utilized because it increases plasma and central progesterone levels akin to that seen during behavioral estrus of rodents, without producing gross alterations in motor behavior and/or coordination [14,16]. After mice were injected with P₄ or vehicle, they were returned to their home cages for 1 h until behavioral testing.

2.5. Behavioral testing

Limited numbers of DATKO mice were available. Given this, a repeated-measures design was utilized. Therefore, all mice were once tested in either the P₄ or vehicle condition, then they were re-tested in the opposite condition five days later. Whether P₄ or vehicle was received initially was counterbalanced across subjects [63,65]. All animals were tested for PPI and for behavior in the activity monitor and open field following vehicle and P₄ conditions.

2.5.1. Handling and habituation—To minimize the effects of handling associated with the repeated testing, on day 1 mice were picked up by the tail and placed back in the home cage. On day 2, mice were transferred to another clean home cage. On day 3, mice were picked up, weighed, and transferred on a cart to another room and then the mouse was moved to another clean home cage. On day 4, the experimenter put the mouse in their home cage on a cart, and mice were transported to another room and were placed in novel environment for 2min. On day 5, mice cages were put in their home cages on a cart, the cart and cage were moved to another room, mice were SC injected with sesame oil, then mice were placed in a novel environment, and then returned to their home cage.

2.5.2. Pre-pulse inhibition—Following the handling period, arousal behavior was assessed in the PPI task. Experiments were conducted based upon previously reported methods [7,17,67]. Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used to measure the startle response. Each chamber consisted of a non-restrictive Plexiglas cylinder mounted on a frame inside a lighted, ventilated box (35cm×35cm×47.5 cm). Movement within the cylinder was detected by piezo-electric accelerometers attached to the cylinder's bottom. Force detected by the accelerometer was converted into analog signals that were digitized and stored electronically. In all experiments, 65 readings were recorded at 1 ms intervals beginning at stimulus onset; the average amplitude was used to describe the acoustic startle response. A high-frequency loudspeaker inside the chamber, mounted above the cylinder, generated broadband background noise and acoustic stimuli, which were controlled by the SR-LAB software system and interface. Sound levels (dB (A)

scale) and accelerometer sensitivity were calibrated routinely, as described previously [7,17]. Mice were tested initially for baseline PPI and pseudo-randomly assigned to hormone treatment groups based on these measurements. Mice were treated with vehicle or P_4 60 min before testing. Experimental sessions consisted of a 5 min acclimatization period with 65 dB broadband background noise followed by PPI sessions. Sessions consisted of five different trial types: no stimulus trials (nostim); startle pulse alone, 40ms duration at 120 dB (p120); and three pre-pulse + pulse trials, 20 ms duration pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12), followed by a 40 ms duration startle stimulus at 120 dB after a 100ms delay. The nostim trial consisted of only background broadband noise. All test sessions started and concluded with six presentations of the p120 trial, while the remainder of the session consisted of 12 presentations of the p120 trial type, 10 presentations of the nostim, the pp3, pp6, and pp12 trial types, in a pseudorandom order, with varying inter-trial intervals (mean 15 s, range 8–23 s). Each animal was tested on a PPI session for 21 min with a total of 64 trials.

2.5.3. Activity monitor—Locomotion was assessed in an activity monitor. Mice were first habituated to the apparatus (40cm×30cm×26cm clear plastic chamber) for 180 min and then subcutaneously injected with P_4 or vehicle. After 1 h, mice were placed back in the apparatus and locomotor activity was measured in 5-min increments using digital counters with passive infrared sensors (Supermex System, Tokyo, Japan).

2.5.4. Open field—Behavior was assessed in the open field, which can be used to determine total motor activity as well as anxiety behavior. Rodents typically avoid open, bright areas so propensity to move in the center of the open field indicates a reduced anxiety-like response. Mice were placed in the open field arena (39cm×39cm×30 cm) that had a 16-square grid floor and an overhead light illuminating the central squares [14,16]. Total numbers of entries and central entries were recorded for 5 min. The total number of entries made in the open field is used as an index of general motor activity, whereas the number of central entries made is an indicator of anti-anxiety-like behavior.

2.6. Statistical analyses

Analyses of variance (ANOVAs) were used to evaluate the effects of the two between-subjects variables (genotype-wildtype or DATKO; sex-male or female), and one-within subject (P_4 or vehicle) variables. Where appropriate, one-way analyses and Fisher's *post hoc* tests were utilized to evaluate groups that were different. The α level for statistical significance was $p < 0.05$.

3. Results

3.1. PPI

There was a main effect of genotype, but neither an effect of sex, nor P_4 , on the magnitude of the startle responses. As has previously been demonstrated, DATKO mice had a diminished startle response following 20 ms duration pre-pulse at 68 dB (pp3) ($F(1,112) = 4.55, p = 0.01$), 71 dB (pp6) ($F(1,112) = 14.07, p = 0.01$), or 77 dB (pp12) ($F(1,112) = 24.29, p = 0.01$), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. See Fig. 1.

3.2. Activity monitor

There were main effects of genotype ($F(1,112) = 72.15, p = 0.01$) and P_4 ($F(1,112) = 3.64, p = 0.05$), but not sex, on the total distance moved in the activity monitor. The interaction between genotype and hormonal status ($F(1,112) = 9.71, p = 0.01$) was attributed to P_4

decreasing the distances DATKO mice traveled, but also tending to increase the distances traveled by the wildtype mice. See Fig. 2.

3.3. Open field

There were main effects of genotype ($F(1,112) = 43.56, p < 0.01$) and P_4 ($F(1,112) = 30.12, p < 0.01$), but not sex, on the total number of entries in the open field. Total entries were lower in wildtype compared to DATKO mice. The interaction between genotype and hormonal status ($F(1,112) = 22.95, p < 0.01$) was due to P_4 decreasing the number of entries of DATKO, but not wildtype, mice. See Fig. 3.

Genotype ($F(1,112) = 56.58, p < 0.01$) and P_4 ($F(1,112) = 36.26, p < 0.01$), but not sex, influenced the number of central entries in the open field. The interaction between genotype and hormonal status ($F(1,112) = 13.57, p < 0.01$) was attributed to P_4 having a much greater effect to increase the number of central entries made by wildtype compared to DATKO mice. See Fig. 4.

4. Discussion

Findings from this study partially supported our hypothesis that progesterone would normalize hyperactivity of DATKO mice. Locomotion in the activity monitor and open field was significantly greater among DATKO, compared to wildtype, mice and P_4 dampened the hyperactivity of DATKO mice. Central entries in the open field were greater among wildtype, compared to DATKO, mice and P_4 significantly increased central entries of wildtype mice. DATKO, compared to wildtype, mice showed a dampened PPI response, but P_4 did not alter this effect. There were no sex differences in these effects. Thus, P_4 had circumspect effects to reduce hyperactivity in the activity monitor and open field of female and male DATKO mice.

The present findings are relevant for previous research that has shown that a prohormone for P_4 , pregnenolone, is lower among schizophrenics, compared to healthy controls and is associated with trait-anxiety scores independent of acute anxiety symptoms [48,56]. Pregnenolone is synthesized during early developmental [20,28,41,49] and, when administered neonatally, influences DA turnover in the striatum [41]. Neonatal pregnenolone-treated rats are considered an animal model of cortical/subcortical dysfunction. Indeed, DA metabolites in the fronto-parietal cortex were similarly increased in pregnenolone-treated female and male rats and resulted in hyperactivity in the open field [42]. These findings and others, in conjunction with the present results that acute P_4 can normalize hyperactivity in DATKO mice, suggest that progestogens may play a role in the pathophysiology of schizophrenia.

One explanation for the effects of progestogens may be due to their effects on stress responses. Schizophrenia is characterized by dysregulation in stress responses. Although diagnosis of schizophrenia is based upon both positive (hallucinations, delusions) and negative symptoms (avolition, alogia) [44,45,57,58,66], there has been a recent emphasis on negative symptoms, which correlate with loss of social function [32], and plasma levels of the stress hormone, cortisol, albeit not P_4 [41–45,58,64,66]. How dysfunction of the hypothalamic-pituitary adrenal (HPA) axis contributes to the pathophysiology of schizophrenia needs to be better understood. Of interest is whether there are differences in HPA responses of DATKO mice compared to their wildtype counterparts in the present study.

One possible mechanism that may underlie some of the effects of progestogens to normalize behavior of DATKO mice are their effects on steroid biosynthesis. This may be particularly

important because no sex differences were observed in the present study, suggesting that there may be a greater role of brain-derived versus ovary/gonad-derived steroids. Neurosteroids, steroid hormones produced in the brain, such as 3 α -hydroxy-5 α -pregnan-20-one (3 α ,5 α -THP), are important endogenous modulators of the HPA that may serve to buffer stress responses. In response to stress, plasma and brain concentrations of 3 α ,5 α -THP are elevated [46,47]. Stress-induced elevations in 3 α ,5 α -THP dampen hyperactivity of the HPA axis [34,52]. 3 α ,5 α -THP has potent agonist-like actions at GABA_A receptors in the brain [52], which underlie its effects to reverse sympathetic activity. 3 α ,5 α -THP from central metabolism of ovarian and/or adrenal P₄ [9] also mitigates stress responses. Although basal levels of 3 α ,5 α -THP are similar for females in the follicular phase and males, during the luteal phase and pregnancy, plasma and hippocampal 3 α ,5 α -THP levels are higher for females than males [25]. Further evidence that 3 α ,5 α -THP mediates HPA responses include that 3 α ,5 α -THP administration to female or male rats attenuates the elevation of plasma adrenocorticotropin (ACTH) and serum corticosterone secretion produced by emotional stress [46]. These data suggest that 3 α ,5 α -THP, produced by glia in response to stress, and/or metabolized in neurons from peripheral sources, may serve as an important mediator of stress responses. It would be of interest to investigate how 3 α ,5 α -THP may be mediating the reduction in hyperactivity that was observed in the present study among DATKO mice administered P₄.

Evidence suggests that 3 α ,5 α -THP may be important in the pathophysiology of schizophrenia. First, neonatal 3 α ,5 α -THP administration to rats disrupts the normal development of the prefrontal cortex and medial dorsal thalamus [18,20], implying that there may be a critical window of vulnerability to neurosteroid insult across development [41,42]. Second, stress-reactivity may underlie the etiology and/or manifestation of schizophrenia. Among people with schizophrenia, dysregulation of the HPA axis is common [33,35,43–45] and stress can precipitate psychiatric episodes related to schizophrenia [45]. Third, stress-induced 3 α ,5 α -THP production can be disrupted in schizophrenia. A novel polymorphism and genetic mutation in the sequence encoding the gene for the mitochondrial benzodiazepine receptor (MBR), which is necessary for 3 α ,5 α -THP biosynthesis in glial cells, has been demonstrated among some schizophrenics, and may create a predisposition to over-sensitivity to stress [34,44]. As well, social isolation (an animal model of schizophrenia) decreases 3 α ,5 α -THP biosynthesis, in the frontal cortex of male Swiss-Webster mice, compared to group-housed controls [6]. Third, there is evidence that 3 α ,5 α -THP metabolized in the brain from peripheral prohormones may reduce the incidence and/or expression of schizophrenia. Women, compared to men, typically have higher levels of 3 α ,5 α -THP, are more likely to have schizophrenia with later onset, better prognosis, and therapeutic response to lower dosages of antipsychotics [23]. When 3 α ,5 α -THP levels are low perimenstrually, first onset, or recurrence of psychotic episodes are more likely and more negative symptoms are reported [21,24,27]. After menopause, when 3 α ,5 α -THP levels are lower, there is a greater recurrence of psychiatric episodes than pre-menopause [8]. Fourth, effective pharmacotherapies for schizophrenia can alter 3 α ,5 α -THP levels. The atypical antipsychotic drug, olanzapine, enhances social functioning and increases 3 α ,5 α -THP levels [13,36]. In an animal model, clozapine can have similar neurosteroidogenic effects; however, it has not been demonstrated to alter circulating neurosteroids levels concomitant with therapeutic effects [41,42]. Together, these data suggest that schizophrenia may involve a reduced capacity to synthesize 3 α ,5 α -THP in the brain, which may increase sensitivity to stress. Although 3 α ,5 α -THP may underlie etiology and/or expression of schizophrenia, the present findings suggest that progestogen-based therapeutics may have a role to normalize the schizophrenic-like behavioral phenotypes [3].

3 α ,5 α -THP may influence the function of the prefrontal cortex (PFC) to mitigate negative symptoms of schizophrenia [30]. Schizophrenia involves PFC hypofunction, poor social

function, and disrupted working memory [32]. The PFC is integral for decisions related to social interactions [12] and working memory, a key component of human reasoning and judgment [32]. Notably, the PFC is sensitive to progestogens. Systemic administration of precursors of 3 α ,5 α -THP enhance working memory [5,12] and 3 α ,5 α -THP enhances dopamine release in the PFC in response to stress [5]. A question is why P₄ did not improve PPI among DATKO mice. It is not that DATKO mice are unresponsive to pharmacotherapies as psychostimulants; NET and SERT inhibitors improve PPI of DATKO mice, and impair PPI of wildtype mice [67]. Given the effects of progestogens for dopamine release in the PFC, it may be that P₄ administration elevated DA in the PFC without the DAT, contributing to a lack of effect of P₄ on PPI among DATKO mice. This may also underlie the apparent (but not significant) effect of P₄ to reduce PPI particularly in the pp3 trial. Further investigation of the potential of hyperdopaminergic action underlying these effects is necessary. Indeed, whether these effects are due to direct actions of progestogens on the PFC or indirect actions of progestogens on the hippocampus and/or VTA, which projects to the PFC, has not been established.

Schizophrenia is characterized by deficits in social functioning and progestogens mediate social behavior in part through actions in the VTA and its projection areas. For example, administration of 3 α ,5 α -THP to the VTA increases time spent in interaction with a conspecific and blocking 3 α ,5 α -THP's formation in the VTA attenuates social behavior. We have also shown that the neurosteroidogenic effects of mating can increase 3 α ,5 α -THP and DA in the midbrain, hippocampus, striatum, and prefrontal cortex. Thus, 3 α ,5 α -THP-enhanced social interactions may involve the VTA and its projections to the mesolimbic dopamine system. A question for future studies is the role of 3 α ,5 α -THP in DATKO and wildtype mice for their social responding.

This is a particularly important area of research. There are differences in plasma levels of pregnenolone, a precursor for 3 α ,5 α -THP, between those with schizophrenia and healthy controls [56]. In a recent study of 21 patients with schizophrenia or schizoaffective disorder, those with the lowest natural levels of pregnenolone, reported the best memory and concentration. In this study, patients took placebo for two weeks and were then randomly assigned to take pregnenolone, as a health supplement, or placebo for eight weeks in conjunction with an antipsychotic. Those taking pregnenolone had about a ~20% reduction in their negative symptoms than did the placebo group [37]. Other studies have also shown that plasma levels of neurosteroids correlate with the severity of negative symptoms among some men with schizophrenia [60]. Thus, it is important to understand further the role of progestogens, such as pregnenolone, P₄, or 3 α ,5 α -THP, given the emerging evidence of their role in the etiology, expression, or treatment of schizophrenia and/or schizoaffective disorders.

Affective, and cognitive, processes are also disrupted in schizophrenia and progestins can influence these behaviors through actions in the hippocampus. In the present study, there was a clear anti-anxiety effect of P₄ among wildtype mice, as demonstrated by an increase in central entries in the open field, independent of an increase in total entries. This same effect was not observed in the DATKO mice, suggesting that there may be some involvement of the DAT for progestogens to increase anti-anxiety responding. However, it may also be that no effects of P₄ were found for central entries of DATKO mice because of the robust effect of P₄ to reduce their motor behavior. A question for follow-up studies would be the effects of P₄ to DATKO and wildtype mice in other typical measures of anxiety behavior of mice (e.g. elevated plus maze, light–dark transition, etc.). As well, whether this effect was due to 3 α ,5 α -THP is of interest. 3 α ,5 α -THP is increased in the hippocampus concomitant with reduced anxiety behavior and enhanced cognitive performance [54,55,65]. Blocking the formation of 3 α ,5 α -THP in the hippocampus increases anxiety behaviors and impairs

cognitive performance [5,11,53]. Given that the hippocampus projects to the PFC, an important question is whether 3 α ,5 α -THP has direct actions in the PFC to mitigate stress and/or behavioral responses. Another possibility is that these effects occur indirectly through connections of the PFC with the VTA and/or striatum. These questions are the topics of ongoing investigation in our laboratory.

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Fig. 1.

The mean (+sem) startle magnitude of male (top panel) and female (bottom panel) mice administered vehicle (open bars) or P₄ (closed bars) before testing at pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$; $n = 15$ /group).

Fig. 2.

The mean (+sem) total distance moved in the Super Mex activity chamber of male (top) and female (bottom) mice administered vehicle (open) or P₄ (closed bars). * Above bar compared to wildtype (left) versus DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15/\text{grp}$).

Fig. 3.

The mean (+sem) total squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P₄ (filled bars). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15/\text{grp}$).

Fig. 4. The mean (+sem) central squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P₄ (filled). * Above bar compared to wildtype (left) and DATKO mice ($p < 0.05$). ** Above bar compared to vehicle ($p < 0.05$; $n = 15/\text{grp}$).