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Hippocampal 3α,5α-THP may alter depressive behavior of pregnant and lactating rats

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Abstract

The 5α-reduced metabolite of progesterone (P), 5α-pregnan-3α-ol-20-one (3α,5α-THP), may mediate progestins' effects to reduce depressive behavior of female rats in part through actions in the hippocampus. To investigate, forced swim test behavior and plasma and hippocampal progestin levels were assessed in groups of rats expected to differ in their 3α,5α-THP levels due to endogenous differences (pregnant and postpartum), administration of a 5α-reductase inhibitor (finasteride; 50 mg/kg sc), and/or gestational stress [prenatal stress (PNS)], an animal model of depression. Pregnant rats had higher plasma and hippocampal 3α,5α-THP levels and less depressive behavior (decreased immobility, increased struggling and swimming) in the forced swim test than did postpartum rats. Finasteride, compared to vehicle administration, reduced plasma and hippocampal 3α,5α-THP levels and increased depressive behavior (increased immobility, decreased struggling and swimming). PNS was associated with lower hippocampal, but not plasma, 3α,5α-THP levels and increased swimming compared to that observed in control rats. Together, these data suggest that 3α,5α-THP in the hippocampus may mediate antidepressive behavior of female rats.

Keywords: Allopregnanolone; Neurosteroids; Nongenomic; Hippocampus; Gestational stress; Affect; Depression; Porsolt

1. Introduction

Progestins may influence depression. Women, compared to men, are twice as likely to experience depression and their depressive episodes are longer lasting and recur more often (Earls, 1987; Nolen-Hoeksema, 1987). From puberty until menopause, women have higher and more labile progestin levels than do men (Pearson Murphy and Allison, 2000). The gender difference in depression emerges during puberty and disappears postmenopause, which suggests that fluctuating progestins [and/or estradiol (E_2)] may have activational effects on mood (Kessler and Walters, 1998; Weissman and Olfson, 1995; Lewinsohn et al., 1998). Women are also uniquely susceptible to mood disorders, such as premenstrual syndrome and/or postpartum depression, that are defined by coincident increases in negative symptomology and changes in endogenous progestin levels (reviewed in Carter et al., 2001; Steiner et al., 2003). Treatments of premenstrual dysphoric disorder that stabilize progestin levels are most effective at improving negative mood symptoms (Freeman et al., 2002; Schmidt et al., 1998). Inducing withdrawal from high, pregnancy-like exposure to estrogen and progesterone (P) produces negative mood symptoms in 62.5% of women with a history of postpartum depression, but did not have an effect on women without a history of postpartum depression (Bloch et al., 2000). Recent evidence suggests that some antidepressant treatments alleviate depressive symptomology coincident with increases in progestin levels in cerebrospinal fluid (Uzunova et al., 1998). Thus, variations in progestins may mitigate the depressive symptomology of some women.

Findings from animal models suggest that progestins can mediate affective behavior of female rodents. To investigate the role of variations in endogenous progestins, depression behavior of female rats across the estrous cycle has been

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examined. Proestrus, a state characterized by high progestin levels in female rodents, is associated with reduced depressive behavior in the forced swim test compared to that seen in male rats or females in other stages of the estrous cycle (Contreras et al., 2000; Frye and Walf, 2002). Proestrous, compared to diestrous, rats demonstrate decreased depressive behavior in the forced swim task concomitant with hippocampal elevations in progestin (but also E_2 and androgen) levels (Frye and Bayon, 1999b; Frye and Walf, 2002; Marvan et al., 1997). A similar pattern of effects is observed with proestrous rats having decreased anxiety behavior, compared to rats in other stages of the estrous cycle that have lower hippocampal progestin (and E_2 and androgen) levels (Frye and Bayon, 1998; Frye et al., 2000). Experimentally decreasing progestin levels in the hippocampus of proestrous rats produces anxiety behavior that is similar to that of diestrous rats (Rhodes and Frye, 2001). These data suggest that endogenous variations in progestins may mitigate some of the changes in affective behavior observed across the estrous cycle.

Although variations in progestins, E_2, and androgens across the cycle may account for estrous cycle changes in affect, and all of these hormones also vary throughout gestation, pregnancy is associated with greater elevations in progestin levels than E_2 or androgen levels (Frye and Bayon, 1999b). However, to date, there has been relatively little investigation of effects of endogenous changes in progestin levels on depressive behavior using comparisons of pregnant and postpartum, lactating rats. There is some evidence of decreased depressive behavior of pregnant rats, compared to ovariectomized (ovx) or postpartum rats, in two depression behavior tasks, the forced swim test and an operant-delayed conditioning task (Molina-Hernandez and Tellez-Alcantara, 2001; Molina-Hernandez et al., 2000). Similarly, increases in depression behavior occur in models of postpartum depression, in which ovx rats have chronic administration of E_2—and P discontinued (Galea et al., 2001). These data suggest that postpartum reductions in progestins may mitigate depression behavior of female rodents.

Progestins' effects on depressive behavior may be due in part to 5α-pregnan-3α-ol-20-one (3α,5α-THP). P is metabolized by the 5α-reductase enzyme to dihydroprogesterone, which is then converted to 3α,5α-THP by actions of the 3α-hydroxysteroid dehydrogenase (3α-HSD) enzyme. P and 3α,5α-THP similarly reduce depressive behavior of rodents. Administration of P or 3α,5α-THP reduces the time rats and mice spent immobile in the forced swim test (Bernardi et al., 1989; Frye et al., 2004b; Khisti and Chopde, 2000; Khisti et al., 2000; Martinez-Mota et al., 1999). P administration would increase P and 3α,5α-THP levels, but 3α,5α-THP would only be expected to increase 3α,5α-THP levels, which suggests that similar behavioral effects produced by P and 3α,5α-THP administration may be attributable to 3α,5α-THP. Notably, other agents that increase 3α,5α-THP, such as antidepressants and neurosteroidogenic drugs, enhance affective behavior (Bitran et al., 2000; Frye and Seliga, 2003; Frye et al., 2003; Serra et al., 2001, 2002; Uzunova et al., 1998; Walf et al., 2004).

In addition to evidence in support of increased 3α,5α-THP levels enhancing affect, decreasing 3α,5α-THP levels has anxiogenic and depressive effects among rodents. Whole brain 3α,5α-THP levels of P-administered ovx mice that are deficient in the 5α-reductase enzyme are lower, and time spent immobile in the forced swim test is increased, compared to that observed for P-administered wild-type mice (Frye et al., 2004b). Similarly, administration of a 5α-reductase inhibitor, finasteride, systemically or to the hippocampus, significantly reduces hippocampal 3α,5α-THP levels and increases anxiety and depressive behavior of proestrous rats compared to that of vehicle-administered rats (Rhodes and Frye, 2001; Frye and Walf, 2002). Precipitating withdrawal from 3α,5α-THP by administering the metabolism inhibitor, indomethacin, increases defensive burying in response to footshock compared to vehicle-administered rats (Gallo and Smith, 1993). The effects of manipulating 5α-reduction in female rats in states characterized by chronic changes in progestin levels, as occurs during pregnancy, on depression behavior have not been thoroughly addressed. Thus, in the present experiment, the effects of systemic finasteride administration on forced swim test behavior and plasma and hippocampal progestin levels of pregnant and postpartum rats were investigated.

There has been some investigation regarding whether changes in 3α,5α-THP produce differences in depression behavior; however, if there are differences in 3α,5α-THP levels produced in animal models of depression is also of interest. In both the learned helplessness and olfactory bulbectomy models of depression, 3α,5α-THP levels are altered in rodents with depressive behavior (Healy and Drugan, 1996; Uzunova et al., 2003).

Gestational stress [prenatal stress (PNS)] is another animal model of depression. There are similarities between the behavioral and physiological characteristics of PNS rats and clinical manifestations of depression (Dugovic et al., 1999; Weinstock, 2001). PNS rats demonstrate behavioral inhibition, such as reduced exploratory behavior, more freezing, and more anxiety/stress-related behavior to novelty than do non-PNS (control) rodents (Drago et al., 1999; Morgan et al., 1999; Overstreet et al., 2000; Pohorecky and Roberts, 1991; Poltyrev et al., 1996; Sternberg, 1999; Sternberg and Ridgway, 2003; Takahashi et al., 1992; Vallee et al., 1997; Wakshlak and Weinstock, 1990; reviewed in Weinstock, 2001; Zimmerberg and Blaskey, 1998). Some behavioral effects in PNS rats may involve differences in the hypothalamic–pituitary–adrenal (HPA) axis of PNS rats. PNS rats have a phase shift in the circadian rhythm of corticosterone secretion (Koehl et al., 1999), increased basal levels of corticosterone (Fride et al., 1986; Peters, 1982; Ward et al., 2000), and elevated levels and/or duration of corticosterone secretion following stress than do control animals (Barbazanges et al., 1996; Henry et al., 1994; McCormick et al., 1995; Peters, 1982; Vallee et al., 1997;

Weinstock et al., 1992). PNS is also associated with cell loss in the hippocampus. PNS female rats have fewer hippocampal granule cells than do controls (Schmitz et al., 2002).

Whether gestational stress is associated with changes in hippocampal 3α,5α-THP concentrations has not been investigated, but there is some evidence to support this. PNS rats respond differently to ovarian hormones compared to non-PNS rats. Performance in tasks of social and depression behavior suggests that PNS rats have decreased responses to endogenous variations in, or exogenous administration of, gonadal hormones than do non-PNS, control female rats (Frye and Orecki, 2002a,b, Frye and Wawrzycki, 2003). Dysregulation of the HPA may underlie some of these behavioral effects of PNS and 3α,5α-THP may mitigate some of these effects by mediating the HPA (Dazzi et al., 1996; Patchev et al., 1994, 1996; Zimmerberg and Brown, 1998; Zimmerberg et al., 1994, 1995, 1999). Notably, an intact hippocampus is necessary for HPA and hypothalamic–pituitary–gonadal (HPG) feedback (Drislane et al., 1994). PNS rats have hippocampal cell loss and 3α,5α-THP has effects to prevent cell loss in the hippocampus (Frye and Bayon, 1999a,b; Frye and Scalise, 2000; Frye et al., 2002; Schmitz et al., 2002; Vongher and Frye, 1999), suggesting PNS rats may have lower hippocampal 3α,5α-THP levels. Furthermore, antidepressant drugs increase neurogenesis in the hippocampus (Duman et al., 2001; Malberg and Duman, 2003) and can also increase 3α,5α-THP levels (Frye et al., 2003, 2004a; Serra et al., 2001, 2002; Uzunova et al., 1998). Thus, whether there are differences in hippocampal 3α,5α-THP levels of PNS and non-PNS rats is of interest.

To investigate the role of 3α,5α-THP in the hippocampus for antidepressive behavior of female rodents, three approaches were used. First, behavior in the forced swim test and 3α,5α-THP levels were compared of lactating rats, 3 days postparturition (postpartum), and in pregnant rats on Gestational Day 18. Second, if decreasing 5α-reduction with systemic administration of finasteride reduced hippocampal 3α,5α-THP levels and increased depressive behavior of rats was investigated. Third, whether PNS and non-PNS, control rats have differences in hippocampal 3α,5α-THP levels and depressive behavior were also determined. We hypothesized that if decreased 3α,5α-THP levels in the hippocampus contribute to increased depressive behavior of female rodents, then rats with lower hippocampal 3α,5α-THP would have increased depressive behavior.

2. Method

These methods were preapproved by the Institutional Animal Care and Use Committee at SUNY-Albany.

2.1. Animals and housing

Female Long–Evans rats ($N = 130$), approximately 55 days old, were obtained from the breeding colony at SUNY-Albany (original stock from Taconic Farms, Germantown, NY). Rats were group housed (4–5 per cage) in polycarbonate cages (45 × 24 × 21 cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. The rats were maintained on a 12:12-h reversed light cycle (lights off 0800 h) with continuous access to Purina Rat Chow and tap water.

2.2. Induction of PNS or control condition

Female rats ($n = 47$) were cycled through two normal estrous cycles (4- to 5-day cycle) and then mated. Fourteen days following mating, pregnant rats were randomly assigned to receive 45 min of restraint stress, under a 60-W light, in a Plexiglas restrainer from Gestational Day 14 to 20 ($n = 25$, stress condition) or no such stress ($n = 22$, control condition). Rats in this nonstressed, control condition remained undisturbed in their home cages. To minimize litter effects, no more than one to two female offspring from each litter were assigned to each experimental group.

2.3. Experimental groups

As adults, rats that experienced PNS ($n = 44$) and rats that did not ($n = 39$) were cycled daily between 0700 and 0800 h to determine those that were in behavioral estrus (Frye and Walf, 2002). Briefly, rats were placed in an arena with a male that was allowed to mount once. If the female responded to the male with a pronounced lordosis posture, the female was considered in behavioral estrus. Rats in behavioral estrus were then mated with a male.

PNS and control pregnant rats were randomly assigned to the pregnant or postpartum condition. Pregnant rats were tested on Day 18 of pregnancy ($n = 44$); of this group, 22 were PNS and 22 were control. Postpartum rats were tested on Day 3 of lactation ($n = 39$); of this group, 22 were PNS and 17 were control.

Rats in each hormonal and stress condition were further randomly assigned to receive vehicle or finasteride before testing. Pregnant, PNS ($n = 13$), pregnant, control ($n = 10$), postpartum, PNS ($n = 12$), and postpartum, control ($n = 8$) were administered vehicle and pregnant, PNS ($n = 9$), pregnant, control ($n = 12$), postpartum, PNS ($n = 10$), and postpartum, control ($n = 9$) rats were administered finasteride.

2.4. Drug administration

Systemic injections of vehicle (subcutaneous; sesame oil and 10% ETOH) or the 5α-reductase inhibitor, finasteride (50 mg/kg sc), were administered to rats 2 h before behavioral testing. Finasteride administration has been shown to reduce plasma and central 3α,5α-THP, but also 5α-reduced androgen, levels (Dazzi et al., 2002; Frye et al., in press(a),(b); Frye and Walf, 2002; Rhodes and Frye, 2001).

2.5. Forced swim task

Rats were tested in a modified version of the forced swim test, a task in which behavior is sensitive to the effects of antidepressant treatment (Frye and Walf, 2002; Frye and Wawrzycki, 2003; Lucki, 1997; Overstreet et al., 1995; Porsolt et al., 1977; Zangen et al., 1997). Rats were placed in a cylindrical container filled with 30 cm of 30 °C water. The depth of water was chosen so that rats would not be able to balance themselves with their tails against the bottom of the cylinder. Water at 30 °C in the forced swim test has been used in our laboratory and others (Chau et al., 2001; Frye and Walf, 2002; Frye and Wawrzycki, 2003; Rada et al., 2003). Water temperature may influence behavior in the forced swim test, such that using lower temperature water (25 °C) reduces body temperature, or produces brain hypothermia, concomitant with immobility in the forced swim test (Abel, 1993; Arai et al., 2000; Taltavull et al., 2003). As well, although the typical paradigm for assessing antidepressants' effects in the forced swim test involves a pretest 24 h prior to testing, a modified version of this task that does not involve a pretest, but rather emphasizes rodents' reactivity to novelty, has been used in our laboratory with success in the past to investigate effects of steroid hormones (Frye and Walf, 2002; Frye and Wawrzycki, 2003). Using a single exposure to the forced swim test also obviates any effects of steroid hormones on memory or cognitive performance in this task (Frye and Lacey, 2000). This paradigm is also validated by data that a tricyclic antidepressant, desipramine, reduces depressive behavior of ovx rats in the forced swim test utilizing the same testing procedure (Walf et al., 2004).

In the forced swim test, the duration spent struggling, swimming, and immobile of rats was recorded for 10 min. Struggling is the quick movement of the front paws that break the surface of the water and attempts to climb, or cling onto, the walls of the testing chamber. Swimming is forelimb and/or hindlimb movement under the surface of the water, where rats do not climb or cling to the side of chamber, and rats' entire body may be completely submerged underwater. Immobility is defined as the absence of any movement (i.e., floating) and is considered an index of depression behavior.

2.6. Tissue collection

Immediately after behavioral testing, some rats in each condition were rapidly decapitated so that tissues could be used to determine plasma and hippocampal P and 3α,5α-THP levels. Whole brains were removed and placed on dry ice and then stored at −70 °C with plasma until radioimmunoassay. Immediately before radioimmunoassay, the hippocampus was dissected out bilaterally, on ice.

2.7. Radioimmunoassay of P and 3α,5α-THP

Plasma and hippocampus P and 3α,5α-THP levels were measured according to previously established methods (Finn and Gee, 1994; Frye and Bayon, 1999a; Frye et al., 1998; Rhodes and Frye, 2001). Briefly, progestins were extracted from plasma with ether and from homogenized brain samples in 50% MeOH and 1% acetic acid. Three hundred microliters of 0.1 M phosphate assay buffer (pH = 7.4) was added to test tubes containing steroid extracts and equilibrated. The P (Dr. G.D. Niswender, Colorado State University, Boulder, CO: #337) or 3α,5α-THP (Dr. Robert Purdy, Veteran's Medical Center, La Jolla, CA: #X-947; #921412-5) antibodies were added to assay tubes with the appropriate tritiated steroids [[^{3}H] P; NET-208: specific activity = 47.5 ci/mmol [^{3}H] 3α,5α-THP; NET-1047, specific activity = 51.3 ci/mmol; New England Nuclear (NEN), Boston, MA]. The standard curve was prepared in duplicate with a range of nine concentrations from 50 to 8000 pg/ml for P and 10–4000 pg/ml for 3α,5α-THP. Incubation at 4 °C for 24 h was terminated by charcoal separation of bound and free. Sample tube concentrations were calculated using the logit–log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery. The intra-assay coefficients of variance were 8% for P and 12% for 3α,5α-THP.

2.8. Statistical analyses

Analyses of variance (ANOVAs) were utilized to examine effects of hormone condition (pregnant or postpartum), finasteride condition (vehicle or finasteride), and gestational stress condition (PNS or control) on behavior in the forced swim test and plasma and hippocampal P and 3α,5α-THP levels. Where appropriate, ANOVAs were followed by Fisher's *post hoc* tests. The α level for statistical significance was $P \leq .05$.

3. Results

3.1. Forced swim test (see Fig. 1 and Table 1)

Postpartum rats had increased depressive behavior in the forced swim test compared to pregnant rats (Fig. 1 and Table 1). Postpartum rats spent significantly more time immobile [$F(1,75) = 33.86$, $P < .01$] and less time struggling [$F(1,75) = 10.90$, $P < .01$] and swimming [$F(1,75) = 20.36$, $P < .01$] than did pregnant rats.

Finasteride increased depressive behavior of female rats. Finasteride, compared to vehicle, significantly increased immobility [$F(1,75) = 10.77$, $P < .01$] and decreased struggling [$F(1,75) = 24.71$, $P < .02$] and swimming [$F(1,75) = 4.54$, $P < .04$].

Although there were no significant main effects of PNS for struggling or immobility behavior, there were effects on swimming duration. PNS rats spent significantly more time swimming than did control rats [$F(1,75) = 4.33$, $P < .04$].

Significant interactions for hormone and finasteride condition were due to finasteride increasing the depressive behavior of pregnant rats. Pregnant, but not postpartum, rats

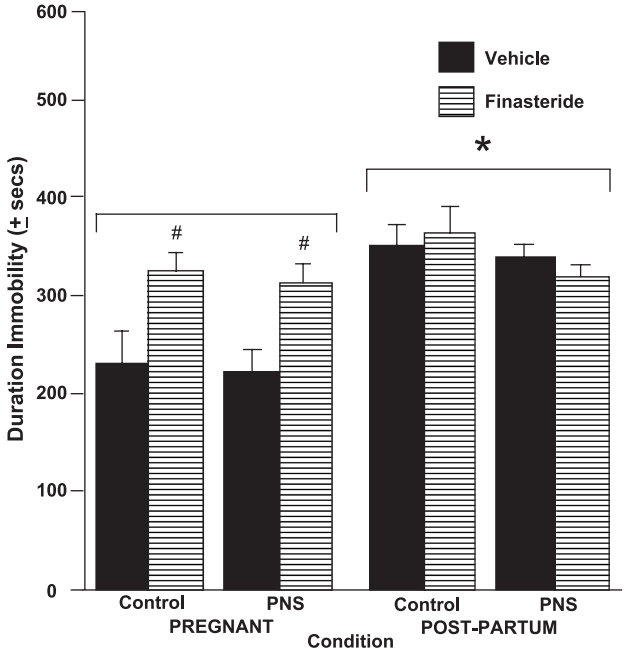

Fig. 1. The mean (± S.E.M.) duration spent immobile in the forced swim test of pregnant and postpartum rats that were PNS or control and administered vehicle (black bars) or finasteride (striped bars) before testing. * Indicates a significant reduction in postpartum, compared to pregnant, rats. # Indicates a significant reduction in finasteride-administered rats compared to vehicle-administered rats.

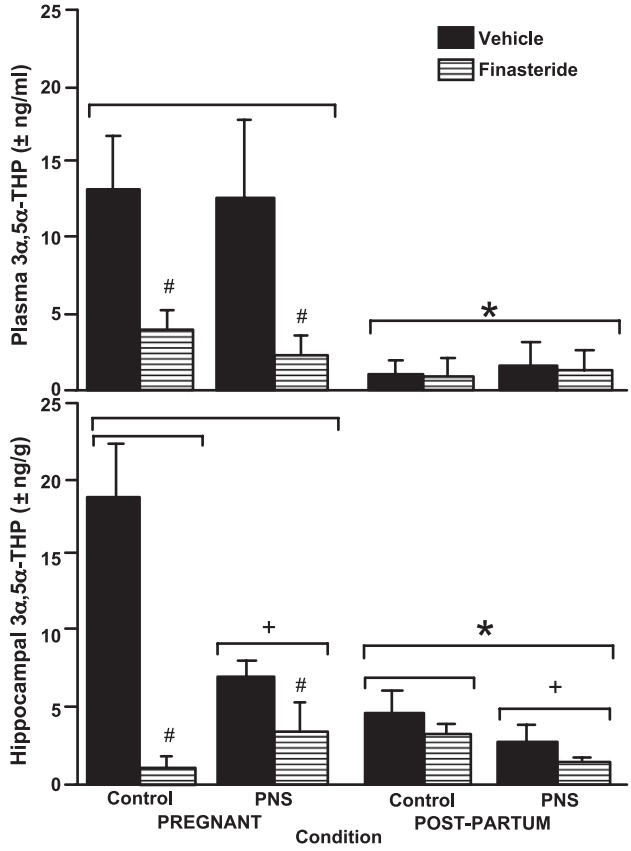

Fig. 2. The mean (± S.E.M.) levels of plasma (top) and hippocampal (bottom) 3α,5α-THP levels of pregnant and postpartum rats that were PNS or control and administered vehicle (black bars) or finasteride (striped bars) before testing. * Indicates a significant reduction in postpartum, compared to pregnant, rats. # Indicates a significant reduction in finasteride-administered rats compared to vehicle-administered rats. + Indicates a significant reduction in PNS rats compared to controls.

administered finasteride spent significantly more time immobile [$F(1,75)=7.77$, $P<.01$] and significantly less time swimming [$F(1,75)=8.07$, $P<.01$].

There was a significant interaction for finasteride and PNS condition for struggling in the forced swim test [$F(1,75)=7.51$, $P<.01$]. Control, but not PNS, rats administered finasteride spent significantly less time struggling.

3.2. Plasma and hippocampal progestin levels (see Fig. 2 and Table 2)

Postpartum rats had significantly lower plasma and hippocampal progestin levels compared to pregnant rats (Fig. 2 and Table 2). Postpartum rats had significantly lower plasma [$F(1,26)=11.12$, $P<.01$] and hippocampal [$F(1,26)=12.96$, $P<.01$] P concentrations than did pregnant rats. There was a similar pattern for postpartum rats to have lower plasma [$F(1,26)=10.79$, $P<.01$] and hippocampal [$F(1,26)=6.15$, $P<.01$] 3α,5α-THP concentrations than did pregnant rats.

Finasteride, compared to vehicle-administration, reduced plasma and hippocampal 3α,5α-THP, but not P, concentrations. Finasteride, compared to vehicle, neither significantly

Table 1
Mean duration (± S.E.M.) of struggling and swimming behavior in the forced swim test

Condition			*n*	Struggling (± s)	Swimming (± s)
Pregnant	Control	Vehicle	10	176.1 ± 9.5	186.2 ± 32.2
		Finasteride	12	123.2 ± 17.1 **	143.2 ± 13.7 **
	PNS	Vehicle	13	141.5 ± 16.6	229.8 ± 15.1***
		Finasteride	9	135.1 ± 14.7 **	150.6 ± 10.0 **,***
Postpartum	Control	Vehicle	8	116.4 ± 12.1 *	101.0 ± 11.5 *
		Finasteride	9	101.3 ± 8.6 *,**	117.2 ± 19.8 *,**
	PNS	Vehicle	12	83.9 ± 19.2 *	134.2 ± 13.8 *,***
		Finasteride	10	116.4 ± 12.1 *,**	135.4 ± 9.3 *,**,***

* Indicates that postpartum rats have significantly reduced struggling or swimming duration than do pregnant rats ($P<.05$).
** Indicates that finasteride-administered rats have significantly reduced struggling or swimming duration than do vehicle-administered rats ($P<.05$).
*** Indicates that PNS rats have significantly reduced swimming duration compared to control rats ($P<.05$).

Table 2
Mean (± S.E.M.) plasma and hippocampal progesterone levels

Condition			n	Progesterone	
				Plasma (ng/ml)	Hippocampus (ng/g)
Pregnant	Control	Vehicle	4	46.8 ± 19.9	36.9 ± 6.8
		Finasteride	5	43.8 ± 16.1	34.9 ± 5.8
	PNS	Vehicle	5	65.8 ± 14.0	36.7 ± 11.4
		Finasteride	4	64.7 ± 13.0	34.0 ± 7.9
Postpartum	Control	Vehicle	3	18.0 ± 1.5 *	20.5 ± 4.9 *
		Finasteride	3	22.7 ± 15.9 *	14.7 ± 5.4 *
	PNS	Vehicle	5	29.8 ± 5.4 *	15.8 ± 4.9 *
		Finasteride	5	25.8 ± 5.1 *	16.2 ± 4.4 *

* Indicates that postpartum rats have significantly lower progesterone levels than do pregnant rats ($P < .05$).

altered plasma, nor hippocampal, P levels. As expected, finasteride significantly reduced plasma [$F(1,26) = 7.42$, $P < .01$] and hippocampal [$F(1,26) = 17.04$, $P < .01$] 3α,5α-THP levels, compared to vehicle.

PNS was associated with lower hippocampal 3α,5α-THP, but not P, levels of rats compared to levels of control rats. Plasma and hippocampal P levels were not significantly different in PNS rats compared to their non-PNS counterparts. Although there was no significant difference in plasma 3α,5α-THP levels of PNS versus control rats, PNS rats did have significantly lower hippocampal 3α,5α-THP levels [4.8 ± 1.1 ng/g; $F(1,26) = 6.15$, $P < .01$] than did control rats (10.1 ± 2.2 ng/g).

There was a significant interaction for hormone and finasteride condition, such that pregnant rats, but not postpartum rats, administered finasteride had significantly lower plasma [$F(1,26) = 6.46$, $P < .01$] and hippocampal [$F(1,26) = 8.55$, $P < .01$] 3α,5α-THP levels.

There was a significant interaction for finasteride and PNS condition on hippocampal 3α,5α-THP levels. Finasteride produced greater reductions in hippocampal 3α,5α-THP levels in control, compared to PNS, rats [$F(1,26) = 8.55$, $P < .02$]. Furthermore, a significant interaction of hormone, finasteride, and PNS condition [$F(1,26) = 6.04$, $P < .02$] such that finasteride significantly reduced hippocampal 3α,5α-THP levels of control, pregnant rats.

4. Discussion

The present results support our hypothesis that lower hippocampal 3α,5α-THP levels would be associated with increased depressive behavior. Postpartum rats, compared to pregnant rats, had lower plasma and hippocampal 3α,5α-THP levels, decreased struggling and swimming, and increased immobility in the forced swim test. Finasteride decreased plasma and hippocampal 3α,5α-THP levels of pregnant rats and reduced struggling and swimming and increased immobility in the forced swim test. PNS rats had lower hippocampal 3α,5α-THP levels and a modest increase in the duration spent swimming. Together, these data suggest that 3α,5α-THP in the hippocampus may be important for modulating depressive behavior of female rats.

Our data that postpartum rats have lower plasma and hippocampal 3α,5α-THP levels and increased depressive behavior in the forced swim test support a role of 3α,5α-THP in the hippocampus for antidepressive behavior of female rodents. Other studies have found similar effects of increased depression behavior in the forced swim and operant-delayed conditioning tasks among ovx and postpartum rats, that would be expected to have low 3α,5α-THP levels, compared to pregnant rats (Molina-Hernandez and Tellez-Alcantara, 2001; Molina-Hernandez et al., 2000). Similarly, immobility in the forced swim task is increased in a model of postpartum depression which involves discontinuation of chronic E_2 and P administration (Galea et al., 2001). However, this pattern has not been reported in all studies. For example, pregnant and nulliparous rats, selectively bred for high- and low-anxiety behavior, had similar behavior (when examined for 90 s) in the forced swim task (Neumann et al., 1998); however, hormonal milieu of nulliparous rats was not accounted for, which may have influenced these results. Overall, the majority of these findings suggest that progestins may have antidepressive effects.

The present findings that finasteride increases depression behavior and reduces plasma and hippocampal 3α,5α-THP levels provide further support that manipulating 3α,5α-THP levels can influence affective behavior. Systemic or intracerebroventricular administration of 3α,5α-THP reduces immobility in the forced swim test of male mice (Hirani et al., 2002; Khisti and Chopde, 2000; Khisti et al., 2000). Increasing endogenous production of 3α,5α-THP in the hippocampus enhances anxiolysis of male rats (Bitran et al., 2000). Furthermore, antianxiety and antidepressive behavior of proestrous rats is attenuated by systemic or intrahippocampal administration of finasteride (Frye and Walf, 2002; Rhodes and Frye, 2001). Thus, altering 3α,5α-THP levels in the hippocampus can influence affective behavior of rats.

The present findings that PNS rats had significantly reduced hippocampal 3α,5α-THP levels suggest that 3α,5α-THP may be modified by gestational stress. Other reports have suggested that some effects of PNS to reduce 3α,5α-THP levels and increase anxiety are reduced by systemic administration of 3α,5α-THP (Zimmerberg and Blaskey, 1998). Although our laboratory has found that this PNS paradigm produces behavioral inhibition and depressive behavior in cycling and ovx rats (Frye and Orecki, 2002a,b; Frye and Wawrzycki, 2003), in this experiment, only subtle differences in forced swim test behavior were observed. 3α,5α-THP may have dampened the HPA response of both PNS and control rats (Dazzi et al., 1996; Patchev et al., 1994, 1996) and behavioral differences in PNS and control rats may not be as apparent in rats with high progestin concentrations associated with pregnancy. Given that repeated exposure to the same, rather than variable, stressors produces less robust gestational stress effects (Kinnunen et al., 2003), it would be

important to ascertain effects on 3α,5α-THP levels produced by a more robust, variable PNS paradigm. Behavioral effects of reduced hippocampal 3α,5α-THP levels, as are seen in the present data, to increase depression behavior may be easier to parse out in rats exposed to a more aversive gestational stress paradigm or those that are acutely stressed immediately before behavioral testing.

Although the findings from the present study are interesting, some limitations to this experiment and interpretation of its results need to be addressed. First, interpretation of PNS effects can be difficult because PNS not only affects the offspring in utero but postnatal maternal behavior towards PNS pups is also altered (Moore and Power, 1985; Power and Moore, 1986; Zimmerberg et al., 2003). Differences in maternal behavior likely influenced the adult behavior of offspring, but these effects are not readily known. We attempted to minimize these effects by using one to two pups from each litter in each experimental group. Second, the possibility that differences between groups in motor behavior may have influenced the behavioral effects of hormonal milieu, gestational stress, and finasteride administration in the forced swim tests needs consideration. For instance, despite dramatically reduced hippocampal 3α,5α-THP levels in pregnant PNS rats, there was little evidence of differences in depressive behavior, such as immobility duration, than that observed in control rats; however, PNS rats had significantly increased swimming duration. Although increased swimming duration does not suggest that PNS rats had altered depressive behavior, it may suggest differences in motor responses. Furthermore, these data also suggest that PNS and 3α,5α-THP manipulations may differentially alter distinct behaviors in the forced swim test (immobility, struggling, and swimming). Antidepressants can differentially alter these behaviors. For instance, serotonin-selective reuptake inhibitors reduce immobility and increase swimming behavior, but antidepressants that increase norepinephrine or dopamine reduce immobility and increase climbing/struggling behavior (Lucki, 1997; Page et al., 1999). Third, the effects of other hormones that vary during pregnancy and postpartum, such as E_2 and androgens, were not addressed. Notably, E_2 and androgens may reduce anxiety and depressive behavior and some stress responses (Bowman et al., 2002, 2003; Estrada-Camarena et al., 2003; Frye and Edinger, 2004; Frye and Seliga, 2001; Frye and Walf, 2004; Frye and Wawrzycki, 2003; Galea et al., 2001; Luine et al., 1998; Osterlund et al., 1999; Rachman et al., 1998; Walf and Frye, submitted for publication; Walf et al., 2004; Young et al., 2001). E_2 also enhances 5α-reductase activity and thereby the production of 3α,5α-THP, which may underlie some of the effects observed (Cheng and Karavolas, 1973; Frye and Duncan, 1996; Sinchak et al., 2003; Vongher and Frye, 1999). Levels of 5α-reduced androgens are also reduced by finasteride administration, or administration of another metabolism inhibitor (indomethacin), which also influences affective behavior (Frye and Edinger, 2004). However, in pregnant rats, levels of 5α-reduced androgens would be much lower than are progestin levels and are thus less likely to produce effects observed in the present study. Fourth, inhibiting activity of the 5α-reductase enzyme reduces both DHP and 3α,5α-THP levels. However, there is little evidence to suggest that DHP reduces anxiety or depressive behavior of rodents.

Whether these behavioral effects of 3α,5α-THP are due to actions at γ-aminobutyric acid ($GABA_A$)/benzodiazepine receptors in the hippocampus was not investigated, but other studies have suggested that 3α,5α-THP's antianxiety effects may occur via $GABA_A$ receptors (Bitran et al., 1991, 1995, 2000; Laconi et al., 2001; Smith et al., 1998; Stock et al., 1999; Wilson, 1992). P and DHP have a high affinity for intracellular progestin receptors (PRs) and 3α,5α-THP has a high affinity for $GABA_A$ receptors, but not PRs (Harrison et al., 1987; Iswari et al., 1986; Majewska et al., 1986; Smith et al., 1974). Thus, it may be important to further investigate the effects and mechanisms, which may involve $GABA_A$/benzodiazepine receptors, of progestins for depression behavior in the future.

In summary, rats with lower hippocampal 3α,5α-THP concentrations had increased depression behavior compared to rats with higher levels. Postpartum rats had significantly lower hippocampal 3α,5α-THP concentrations and more depressive behavior than did pregnant rats. Finasteride, compared to vehicle, administered to pregnant and postpartum rats, significantly reduced plasma and hippocampal 3α,5α-THP levels and increased depressive behavior in the forced swim test. Furthermore, PNS rats also had lower hippocampal 3α,5α-THP levels and modest differences in the forced swim test. Together, these data suggest that P's metabolism to 3α,5α-THP in the hippocampus may be important for antidepressive behavior.

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