Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 78 (2004) 473–481

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats

Cheryl A. Frye[a,b,c,*], Kassandra L. Edinger[a,b]

[a]Department of Psychology, State University of New York at Albany, 1400 Washington Avenue, Albany, NY 12222, USA
[b]Department of Biological Sciences, State University of New York at Albany, 1400 Washington Avenue, Albany, NY 12222, USA
[c]The Center for Neuroscience Research, State University of New York at Albany, 1400 Washington Avenue, Albany, NY 12222, USA

Received 18 March 2004; received in revised form 31 March 2004; accepted 13 April 2004

Abstract

Androgens may mediate anxiety behaviors; however, these effects and mechanisms of androgens are not well understood. The following experiments investigated whether testosterone (T)'s effects on anxiety behavior are mediated by its 5α-reduced, nonaromatizable metabolite dihydrotestosterone (DHT) and/or its 3α-hydroxysteroid dehydrogenase (3α-HSD) reduced metabolite 3α-androstanediol (3α-diol). In Experiment 1, gonadally-intact adult male rats and gonadectomized (GDX), DHT-replaced rats had similar low levels of anxiety behavior in the open field and elevated plus maze and fear behavior in the defensive freezing task compared with GDX control rats. In Experiment 2, intact or DHT-replaced rats that received blank inserts to the hippocampus demonstrated less anxiety behavior than did rats administered an implant of indomethacin, a 3α-HSD inhibitor, to the dorsal hippocampus. These data indicate that T's 5α-reduced metabolite, DHT, can reduce anxiety behavior and that blocking metabolism to 3α-diol in the hippocampus can attenuate these effects.

Keywords: Androgens; Nongenomic; $GABA_A$; Androgen receptors; Affect

1. Introduction

Androgens may mediate anxiety behaviors among rodents. Acute (a high dose of testosterone [T] 24 h before testing) or chronic (high levels of T for 8 weeks) T administration to intact male rats increases punished drinking in the Vogel paradigm (Bing et al., 1998). T priopionate via Silastic capsules for 1 weeks, but not 2 weeks, increase exploration of intact rats in the plus maze (Bitran et al., 1993). As well, acute endogenous T release, produced by sexual stimuli, can also increase exploration by intact mice in the plus maze (Aikey et al., 2002). Removal of the testes (gonadectomy; GDX), the primary source of androgens, increases anxiety behavior in several tasks. GDX rodents have decreased activity in the center squares of the open field, and spend more time freezing or burying in response to shock than do intact male rats (Adler et al., 1999; Bitran et al., 1993, Fernandez-Guasti and Martinez-Mota, 2003; Frye and Seliga, 2001). Androgen-replacement to GDX rats can reverse the effects of GDX on behavior in the open field or in response to shock (Fernandez-Guasti and Martinez-Mota, 2003; Frye and Seliga, 2001). Thus, T may produce exposure-dependent effects on anti-anxiety behavior in animal models.

Metabolites of T may mediate some of androgens' influence on anxiety. Separate reports indicate that replacement with T or its nonaromatizable, 5α-reduced metabolite dihydrotestosterone (DHT) to GDX rats similarly decrease anxiety behavior (Frye and Seliga, 2001; Frye et al., in press). DHT can be further reduced by the 3α-hydroxysteroid dehydrogenase enzyme (3α-HSD) to 3α-androstanediol (3α-diol). 3α-Diol administration can decrease anxiety behavior of intact male rats (Bitran et al., 1996) or ovariectomized female rats (Frye and Lacey, 2001). These findings support the idea that androgens effects on anxiety behavior may require 5α-reduction rather than aromatization to estrogen (E_2), which can also decrease anxiety and/or depression

* Corresponding author. Department of Psychology, State University of New York at Albany, 1400 Washington Avenue, Albany, NY 12222, USA. Tel.: +1-518-442-4836; fax: +1-518-442-4867.
E-mail address: cafrye@cnsunix.albany.edu (C.A. Frye).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.04.019

behavior of female rats (Walf and Frye, submitted for publication; Walf et al., 2004).

The hippocampus is a putative central site of androgen's actions to mediate anxiety behavior. The hippocampus is important for anxiety behavior (Bannerman et al., 2002) and androgens can have actions in the hippocampus. Tritiated T is taken up in the hippocampus of rats (Pfaff, 1968). Although castration reduces neuronal firing, increases vulnerability to cell death, and reduces synapse density in the hippocampus, androgen-replacement reverses these effects (Frye and McCormick, 2000a,b; Isgor and Sengelaub, 2003; Leranth et al., 2003; Mizoguchi et al., 1992; Pouliot et al., 1996; Smith et al., 2002). All of the enzymes necessary for T's metabolism, aromatase, 5α-reductase, and 3α-HSD, are located within the hippocampus (Ivanova and Beyer, 2000; Jacobs et al., 1999; Li et al., 1997), such that T metabolites are readily formed in this region (Li et al., 1997; MacLusky et al., 1994; Pelletier et al., 1994). These data suggest that the hippocampus is a target of T and its metabolites.

The present experiment examined the influence of 5α-reduced androgens in the hippocampus on anxiety behavior. We hypothesized that if actions of 5α-reduced androgens are sufficient and E_2 is not necessary for androgen-mediated antianxiety behavior, then the anxiety behavior of intact and GDX rats administered the nonaromatizable androgen, DHT, should be similar and less than that of GDX control rats. If formation of 3α-diol is necessary for androgen-mediated antianxiety behavior, then blocking DHT's metabolism to 3α-diol with indomethacin, a 3α-HSD blocker, would reduce DHT's antianxiety behavior.

2. Methods

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

2.1. Animals and housing

Male Long–Evans rats ($N=105$), approximately 55 days of age, were obtained from the colony at the University at Albany (original stock from Taconic Farms, Germantown, NY). Rats were group housed in polycarbonate cages (45 × 24 × 21 cm), in a temperature- (21 ± 1 °C) and humidity- (45–55%) controlled room that was maintained on a 12/12 reversed light cycle (lights off at 8:00 a.m.). Rats had unrestricted access to Purina Rat Chow and tap water in their home cages.

2.2. Surgery and androgen replacement

2.2.1. Androgen regimen

Rats were anesthetized with Rompun (60 mg/kg) and Ketaset (80 mg/kg) and were randomly assigned to sham surgery (intact, $n=35$) or GDX ($n=70$), 4–6 weeks prior to androgen replacement and behavioral testing. GDX rats were randomly assigned to receive a single subcutaneous Silastic implant (1.57 mm inner diameter, 3.18 mm outer diameter) that was either left empty ($n=34$) or contained crystalline DHT ($n=36$; Sigma, St. Louis, MO; 10 mm per animal), 48 h prior to the first testing occasion. This DHT regimen provides a sustained release of DHT and produces physiological levels of DHT and 3α-diol in plasma and in the hippocampus (Edinger and Frye, in press).

2.2.2. Stereotaxic surgery

Four to 6 weeks following GDX and 1 week prior to testing, rats in Experiment 2 ($n=75$) were stereotaxically implanted with bilateral guide cannulae aimed at the dorsal hippocampus (from bregma AP = −3.8, ML = ±2.0, DV = −2.0; Paxinos and Watson, 1986). Cannulae consisted of 23-gauge thin-wall stainless steel guide tubing with 30-gauge removable inserts, designed to extend 3 mm beyond the edge of the guide cannulae. The dorsal hippocampus was targeted because infusions of T and its metabolites to this region alter anxiety behavior of GDX rats (Edinger and Frye, in press) and there are high levels of metabolism enzymes in this region (Rhodes and Frye, 2004).

2.2.3. Inserts for guide cannulae

Inserts for the guide cannulae were tamped in 1 μg of indomethacin or left empty (vehicle control). This results in 1 μg of indomethacin in the base of the insert. The indomethacin-containing inserts were examined with a dissecting microscope prior to implantation to verify that indomethacin was only present within the lumen of the insert. Inserts were placed into the guide cannulae 2 h prior to testing on three separate occasions, with a minimum of 48 h between administrations. Rats were randomly assigned to either indomethacin or control condition at the beginning of the experiment and were tested in that same condition on all occasions. Hippocampal inserts were removed immediately following testing in each task. This regimen has been previously demonstrated to reduce 3α-diol levels in the hippocampus of intact and/or DHT-replaced male rats to that of GDX rats (Frye et al., in press).

2.3. Behavioral testing

Separate groups of rats were used for testing in Experiments 1 and 2. All rats were tested on three occasions, once in the open field, once in the elevated plus maze, and once in the defensive freezing task. Testing for each task occurred on a separate day, with a minimum of 48 h between testing in each task. The order of the tasks was counterbalanced to minimize order effects. Rats were tested by one of three investigators uninformed of the hypothesized outcomes. There was greater

than 95% concordance between the observations of the three investigators.

2.3.1. Open field

The open field task is a measure of anxiety behavior (Frye et al., 2000) and was employed according to previously published methods (Frye et al., 2000; Pellow et al., 1984). Rats were placed in the corner of the open field box (76 × 57 × 35), which is marked off into a 48-square grid. Rats were observed for 5 min and the number of central and outer squares entered was recorded.

2.3.2. Elevated plus maze

The elevated plus maze was used as a measure of anxiety behavior (Frye et al., 2000; Pellow and File, 1986). The elevated plus maze has two open arms and two arms enclosed with 30-cm-high walls. Arms measure 49 × 10 cm and are elevated 50 cm off the ground. Rats were placed at the junction of the arms, and time spent on and number of entries to the open and closed arms were recorded for 5 min.

2.3.3. Defensive freezing

The defensive freezing task was used as a measure of fear behavior (Frye et al., 2000; Gallo and Smith, 1993; Treit, 1984). Rats were placed in a clear, Plexiglas testing chamber (26.0 × 21.2 × 24.7 cm) containing an electrified probe (2.5 cm in diameter and 10.0 cm in height), located 3.0 cm from the back wall and 2.5 cm from the right wall. Wood chip bedding was placed on the floor of the chamber so that the pedestal extended 4.5 cm above the bedding. The pedestal was wrapped in two wires that were connected to the source (Lafayette Model A615B, Lafayette, IN) set to deliver 6.66 mA of unscrambled shock when the animal touched the pedestal with its forepaws. The shock was terminated, and the time spent freezing or burying after shock was observed for 15 min. The height of the sawdust chips, in the corner of the chamber near the prod, was also measured after behavioral testing.

2.4. Perfusion and histological analyses

To determine insert locations aimed at the hippocampus, rats were exsanguinated with 0.9% saline followed by fixation with 10% formalin by intracardial gravity perfusion. Brains were frozen, sliced at 40 μm on a cryostat, and stained with Cresyl violet. Insert locations were visualized by light microscopy.

2.5. Site analyses

The data from three rats that had inserts not in the CA1 region of the dorsal hippocampus were excluded. We have previously determined the region of effective intrahippocampal androgen inserts that produce antianxiety behavior is a circumscribed area of the dorsal hippocampus that encompasses both the entorhinal cortex and subiculum. The extent of the spread of such implants was examined previously by including a small amount of Cresyl violet dye in androgen-containing inserts. Using this approach, we found a spread of 1–2 mm from the insert site. In this experiment, as in previous studies with application of androgens to the hippocampus (Edinger and Frye, in press), rats with indomethacin inserts aimed at the dorsal hippocampus that were not actually in this region had behavior similar to animals that had control implants to the correct site. The data of three rats that had inserts that were too ventral and were in the posterior nucleus of the hypothalamus, rather than the hippocampus, are presented in Table 1.

2.6. Procedures

2.6.1. Experiment 1

To examine the effect of androgens on anxiety and fear behavior, rats were randomly assigned to 1 of 3 groups: intact ($n = 10$), GDX ($n = 10$), or GDX and DHT-replaced ($n = 10$). Intact, GDX, and DHT-replaced rats were tested in the open field, elevated plus maze, and defensive freezing tasks.

2.6.2. Experiment 2

To examine the effects of inhibiting 3α-HSD, the anxiety and fear behavior of other intact, GDX, or GDX and DHT-replaced rats was examined. Rats in each androgen condition received indomethacin, a 3α-HSD inhibitor, or blank inserts ($n = 12$ per six groups) aimed directly to the hippocampus, 2 h prior to three separate testing occasions (once in the open field, elevated plus maze, and defensive freezing task).

2.7. Statistical analyses

For Experiment 1, one-way analyses of variances (ANOVAs) were used to examine the differences between groups. For Experiment 2, two-way ANOVAs were used to examine effects of androgen and hippocampal implant conditions on behavior. The α level for statistical significance was $P \leq .05$. Where appropriate, Fisher's *post hoc*

Table 1
Anxiety behavior in rats with inserts not corresponding to the CA1 region of the dorsal hippocampus ($n = 3$)

Androgen condition	Intact	DHT-replaced	
Drug condition	Indomethacin	Vehicle	Indomethacin
Number of central entries in open field	22	9	17
Open arm time (s) in elevated plus maze	45	9	20
Duration freezing (s) in defensive freezing	302	444	258

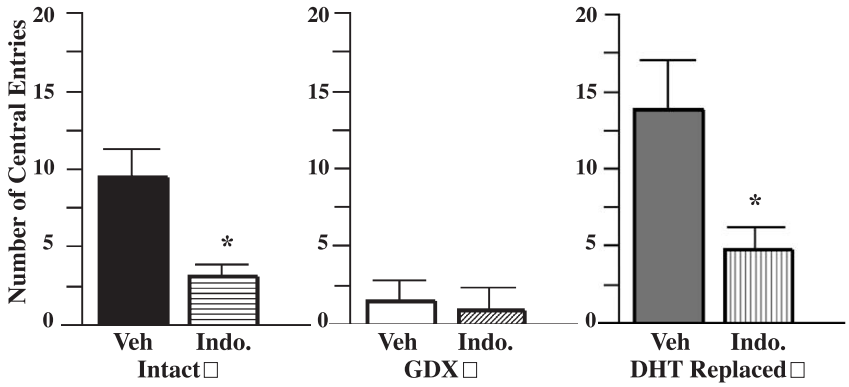

Fig. 1. The left panel represents the mean central entries (± S.E.M.) of intact rats with blank inserts (vehicle; black bar) or 1 μg of indomethacin (Indo.; striped black bar) to the hippocampus. The middle panel represents the mean central entries (± S.E.M.) of GDX rats with intrahippocampal vehicle (white bar) or indomethacin (striped black bar) The right panel represents the mean central entries (± S.E.M.) of DHT-replaced rats with intrahippocampal vehicle (gray bar) or indomethacin (striped gray bar). * Significant difference ($P < .05$) between control implants and hippocampal indomethacin for this androgen condition ($n = 12$ per group).

tests were used to determine groups that were different. A trend was considered $P \leq .10$.

3. Results

3.1. Open field

3.1.1. Experiment 1

Intact and DHT-replaced rats were more active in the open field than were GDX rats. Significantly more central entries [$F(2,27) = 4.35$, $P = .02$] were made by intact (10.4 ± 2.2) and DHT-replaced (13.6 ± 2.6) than GDX (4.7 ± 1.3) rats. Intact (121.7 ± 10.3) and DHT-replaced rats (86.0 ± 10.9) made more entries into the peripheral squares of the open field [$F(2,27) = 8.60$, $P = .001$] than did GDX (48.1 ± 15.7) rats.

3.1.2. Experiment 2

There were main effects of androgen [$F(2,66) = 12.01$, $P = .001$] and hippocampal implant [$F(1,66) = 16.39$, $P = .001$] condition on the number of central entries in the open field. Intact and DHT-replaced rats made more central entries than did GDX rats. Indomethacin reduced central entries compared with those without implants. The interaction [$F(2,66) = 3.84$, $P = .02$] between these conditions was due to indomethacin-decreasing central entries of intact and DHT-replaced, but not GDX, rats (see Fig. 1). There were main effects of androgen condition to increase the number of peripheral [$F(2,66) = 8.89$, $P = .01$] squares entered, which was again due to intact (84.8 ± 12.3) and DHT-replaced (115.3 ± 12.2) rats making more entries to the peripheral squares than did the GDX rats. There were neither main effects of hippocampal implant condition, nor an interaction between androgen

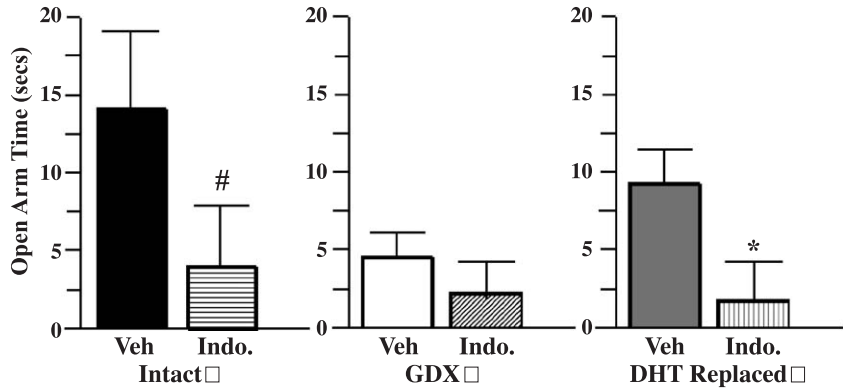

Fig. 2. The left panel represents the mean open arm time (± S.E.M.) of intact rats with blank inserts (vehicle; black bar) or 1 μg of indomethacin (Indo.; striped black bar) to the hippocampus. The middle panel represents the mean open arm time (± S.E.M.) of GDX rats with intrahippocampal vehicle (white bar) or intrahippocampal indomethacin (black striped bar). The right panel represents the mean open arm time (± S.E.M.) of DHT-replaced rats with intrahippocampal vehicle (gray bar) or indomethacin (striped gray bar). * Significant difference ($P < .05$) and $^{\#}$tendency for a difference ($P < .10$) between control implants and hippocampal indomethacin for the androgen condition indicated ($n = 12$ per group).

Table 2
Elevated plus maze behavior in intact, GDX, and DHT-replaced rats that received blank inserts (vehicle) or 1 μg of indomethacin ($n = 12$ per group), a 3α-HSD inhibitor, to the hippocampus

Androgen condition	Intact		GDX		DHT-replaced	
Drug condition	Vehicle	Indomethacin	Vehicle	Indomethacin	Vehicle	Indomethacin
Number of open arm entries	1.6 ± 0.4 *	0.7 ± 0.4	0.5 ± 0.2	0.4 ± 0.3	1.08 ± 0.2	0.2 ± 0.1 *
Number of closed arm entries	7.83 ± 0.8	7.08 ± 0.9	2.3 ± 0.4	2.6 ± 0.6	5.6 ± 0.6	6.75 ± 0.9
Closed arm time (s)	284.2 ± 3.7	295.3 ± 3.7	295.4 ± 1.7	297.7 ± 1.9	291.2 ± 2.3	298.2 ± 4.1 *

* Significant difference between control group and indomethacin for the androgen condition indicated ($P < .05$).

condition and implant condition in the number of peripheral squares entered.

3.2. Elevated plus maze

3.2.1. Experiment 1

Intact and DHT-replaced rats were more exploratory in the elevated plus maze than were GDX rats. Intact (14.0 ± 3.1 s) and DHT-replaced (13.6 ± 5.2 s) rats spent significantly longer on the open arms of the elevated plus maze [$F(2,27) = 3.74$, P=.04] than did GDX (2.0 ± 0.6 s) rats. Intact (3.5 ± 0.4) and DHT-replaced (5.8 ± 0.8) rats made significantly more closed arm entries [$F(2,27) = 15.45$, P=.001] compared with GDX rats (0.9 ± 0.5). The number of open arm entries was not different among intact (1.4 ± 0.4), DHT-replaced (1.2 ± 0.3), and GDX (1.9 ± 1.5) rats. As well, the amount of time spent on the closed arms was similar for intact (286.8 ± 3.5 s), DHT-replaced (287.4 ± 5.4 s), and GDX (296.4 ± 17.1 s) rats.

3.2.2. Experiment 2

The main effect of androgen condition on open arm time in the elevated plus maze approached significance [$F(2,66) = 2.92$, P=.06]. The main effect of hippocampal implant [$F(1,66) = 8.62$, P=.004] was due to indomethacin reducing the amount of time spent on the open arms compared with control implants. Although there was no significant interaction between androgen and hippocampal condition, indomethacin administration decreased open arm time of intact and DHT-replaced, but not GDX, rats (see Fig. 2). As Table 2 illustrates, there was a tendency for a main effect on androgen [$F(2,66) = 2.71$, P=.07], and a significant main effect on hippocampal implant [$F(1,66) = 8.25$, P=.005] on the number of open arm entries made. Intact and DHT-replaced rats made more open arm entries, and indomethacin reduced this effect. There was a main effect of androgen on closed arm time [$F(2,66) = 3.21$, P=.04] and closed arm entries [$F(2,66) = 24.289$, P=.001]. Intact and DHT-replaced rats spent less time on the closed arms and made more entries into the closed arms than did GDX rats.

3.3. Defensive freezing

3.3.1. Experiment 1

Intact and DHT-replaced rats show less fear behavior in the defensive freezing task than did GDX rats. Intact (305.0 ± 47.0 s) and DHT-replaced (334.3 ± 33.7 s) rats spent significantly less time freezing in response to shock compared with GDX (636.6 ± 81.4 s) rats [$F(2,27) = 10.13$, P=.005]. Intact (55.5 ± 24.2 s), DHT-replaced (72.0 ± 26.1 s), and GDX (64.2 ± 37.7 s), rats spent similar amounts of time burying in response to shock. Intact (3.5 ± 0.4), DHT-replaced (3.4 ± 0.4), and GDX (2.9 + 0.3) rats produced sawdust of similar height in response to shock.

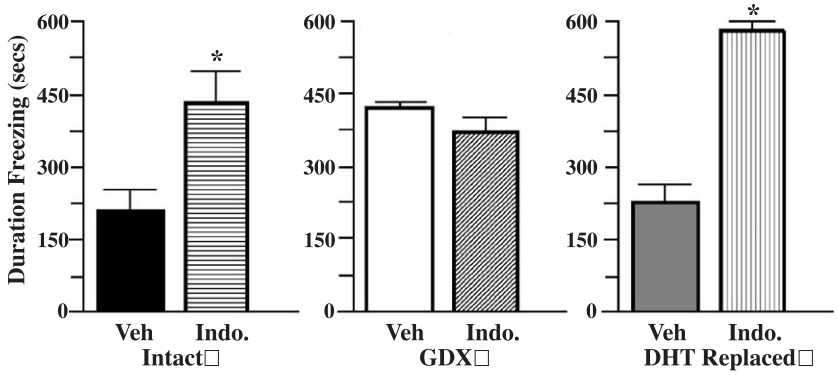

Fig. 3. The left panel represents the mean freezing time (± S.E.M.) of intact rats with blank inserts (vehicle; black bar) or 1 μg of indomethacin (Indo.; striped black bar) to the hippocampus. The middle panel represents the mean freezing time (± S.E.M.) of GDX rats with intrahippocampal vehicle (white bar) or indomethacin (black striped bar). The right panel represents the mean freezing time (± S.E.M.) of DHT-replaced rats with intrahippocampal vehicle (gray bar) or indomethacin (striped gray bar). * Significant difference ($P < .05$) between control implants and hippocampal indomethacin for the androgen condition indicated ($n = 12$ per group).

Table 3
Defensive freezing behavior in intact, GDX, and DHT-replaced rats that received blank inserts (vehicle) or indomethacin ($n = 12$ per group), a 3α-HSD inhibitor, to the hippocampus

Androgen condition	Intact		GDX		DHT-replaced	
Drug condition	Vehicle	Indomethacin	Vehicle	Indomethacin	Vehicle	Indomethacin
Duration burying (s)	70.75 ± 23.9	63.17 ± 18.2	3.9 ± 2.7	34.3 ± 19.3	173.7 ± 42.1 *	100.5 ± 28.3
Sawdust height (cm)	10.2 ± 0.5	6.8 ± 0.09 *	2.6 ± 0.05	3.0 ± 0.3	12.8 ± 0.5	13.4 ± 0.4

* Significant difference between indomethacin and control implants for the androgen condition indicated, and a significant difference across androgen conditions ($P < .05$).

3.3.2. Experiment 2

There was a tendency for androgen condition to influence behavior [$F(2,66) = 2.41$, $P = .09$]. Intact and DHT-replaced rats spent less time freezing. There was a main effect of hippocampal implant condition [$F(1,66) = 31.98$, $P = .001$] on duration of freezing in response to shock. Indomethacin increased freezing. The interaction between these variables [$F(2,66) = 15.44$, $P = .001$] is attributable to indomethacin increased freezing of intact and DHT-replaced, but not GDX, rats (see Fig. 3). There were main effects of androgen [$F(2,66) = 13.049$, $P = .001$] and a tendency of hippocampal condition [$F(1,66) = 2.64$, $P = .09$] to influence duration of burying in response to shock (see Table 3). Intact and DHT-replaced rats spent more time burying, and indomethacin decreased this burying behavior. There was a trend for an interaction between these variables [$F(2,66) = 2.64$, $P = .08$], which is associated with indomethacin increasing burying of GDX rats, but decreasing burying of DHT-replaced rats. Androgen [$F(2,66) = 27.00$, $P = .001$], but not hippocampal condition, influences dust height. Intact and DHT-replaced rats produced greater dust heights than did GDX rats.

4. Discussion

The results of these experiments supported our hypotheses. First, we hypothesized that if T's aromatization to E_2 was not necessary for the effects of androgens to mediate anxiety behavior, then replacement with the nonaromatizable androgen, DHT, would result in similar anxiety behavior, as was observed for intact rats, which would be greater than that of GDX. Rats subcutaneously replaced with DHT showed anxiety behavior in the open field, elevated plus maze, and defensive freezing tasks, that was similar to that of intact rats, and significantly less than that of GDX rats. Second, we hypothesized that if metabolism to 3α-diol was important for anxiety behavior, then blocking DHT's metabolism to 3α-diol with indomethacin would increase anxiety behavior in the open field and elevated plus maze tasks and fear behavior in the defensive freezing task of intact and DHT-replaced, but not GDX, rats. In support, intrahippocampal indomethacin administration to intact and DHT-replaced rats significantly decreased central entries to a brightly lit open field, amount of time spent on the open arms of the elevated plus maze, and the duration of freezing in response to shock. Together, these data suggest that 5α-reduced metabolites in the hippocampus may mediate anxiety behaviors.

The present findings confirm and extend previous research that suggests that androgens may mediate anxiety behaviors of rodents. First, the present results, as in previous open field and defensive freezing experiments (Adler et al., 1999; Bitran et al., 1993; Frye and Seliga, 2001), confirm that androgen replacement can reinstate antianxiety behavior that was altered by GDX. Second, the present experiment extends these findings with the use of a nonaromatizable androgen, DHT. The anxiety or fear behavior of intact and DHT-replaced rats was similar, which indicates that aromatization to E_2 is not necessary for these androgen-mediated anxiety behaviors in these tasks. Third, this experiment suggests that the hippocampus is an important brain region for 5α-reduced androgens' effects on anxiety and fear behaviors. Indeed, indomethacin to the hippocampus increased anxiety behavior in the open field and elevated plus maze and fear behavior in the defensive freezing task.

Although the findings of this experiment are interesting, it is necessary to use caution when interpreting these data. For example, while DHT-administered and intact rats had similar anxiety levels in the number of central squares entered in the open field and the duration freezing in the defensive burial task, there were differences between these groups in the duration of burying behavior (in the second but not first experiment). DHT-administered rats spent significantly longer burying in response to shock, which is also considered an index of anxiety (Gallo and Smith, 1993). Notably, indomethacin had no effect on this measure of anxiety, but it increased anxiety behavior in the open field and plus maze and freezing in response to shock. Previous reports indicate that intact or T-replaced rats spend significantly less time burying in response to shock in the defensive freezing paradigm (Fernandez-Guasti and Martinez-Mota, 2003; Frye and Seliga, 2001). We have found that freezing, rather than burying behavior, shows more consistent and robust effects. Treit et al. (1986) has proposed that the shock intensity in this paradigm influences animals' behavioral responses such that intense shock elicits freezing. Given the 6.6-mA shock intensity versus the 0.3-mA shock intensity used by others (Gallo and Smith, 1993), it is possible that differences in our observations and others for burying and freezing represent active versus passive fear

behavior (DeBoer and Koolhaas, 2003). The differences in burying we have observed between studies may be a result of the small amount of burying behavior occurring during the 15-min observation period (Frye and Seliga, 2001), which has led to us to focus on other behavioral responses in response to shock, such as freezing. These findings suggest that androgens may have task-dependent effects on anxiety behavior. As well, while the pattern of effects in the plus maze was consistent with the other behavioral assays, effects in this paradigm were modest, suggesting antianxiety rather than anxiolytic effects.

While these findings indicate that DHT and 3α-diol may mediate androgens' influence on anxiety behavior through actions in the hippocampus, there may be concerns that indomethacin's effects to alter anxiety and fear behavior were not specifically due to decreasing 3α-diol formation in the hippocampus but rather attributable to other effects of indomethacin. Although *systemic* indomethacin has been reported to produce nonspecific effects that can make rats sick and indirectly reduce behavior by decreasing locomotion (Beyer et al., 1999), the central manipulations with indomethacin neither produced nonspecific behavioral effects in this experiment nor in previous experiments involving its central administration (Frye and Walf, 2002, 2004). For example, indomethacin administration had no effect on the number of peripheral squares entered in the open field or the number of closed arm entries in the elevated plus maze. While indomethacin decreased burying behavior in DHT-replaced rats, there was no effect of indomethacin on burying behavior in intact or GDX rats. Together, these findings suggest that intrahippocampal indomethacin regimen may not influence motor behavior in the anxiety tasks used. A related point is that this indomethacin regimen, which has been previously demonstrated to decrease 3α-diol formation and 3α-diol-mediated behaviors (Frye et al., in press), also decreases other 5α-reduced steroids, such as allopregnanolone, which can also alter anxiety behaviors (Frye and Walf, 2002, 2004; Frye et al., 2000). Throughout development, males have much lower levels, and less variation in, 5α-reduced progestins and its precursors, produced by the gonads and the adrenals, than do females (Paul and Purdy, 1992). This suggests indomethacin here likely had more salient effects to alter androgenic rather than pregnane metabolites. However, central production of allopregnanalone also varies in response to stress (Dazzi et al., 1996, 2002; Reilly et al., 2000; Zimmerberg and Brown, 1998). Potential effects of indomethacin on stress-induced production of allopregnanalone, its precursors, or other neurosteroids, which could have influenced the results of these experiments, cannot be obviated. Indomethacin can also increase prostaglandin metabolism (Clunie et al., 2003), which can enhance fear and/or anxiety responses in people and rodents (Amateu and McCarthy, 2002; Clunie et al., 2003; Oka et al., 2001; Song et al., 2003). Some of indomethacin's effects to enhance anxiety may be due to its effects on prostaglandins. As well, indomethacin can also inhibit cyclooxygenase-2. Future experiments should compare effects of a specific cyclooxygenase-2 inhibitor to that of indomethacin and finasteride (another 5α-reductase inhibitor) on anxiety behavior to rule out these potential nonspecific effects of indomethacin. Regarding the former and latter points, if indomethacin produced nonspecific increases in anxiety behavior, then indomethacin would be expected to increase anxiety behavior of GDX rats. Notably, indomethacin increased burying by GDX rats, but did not increase anxiety behavior in the open field and elevated plus maze, suggesting that it did not have pervasive, indiscriminant, anxiogenic effects. Albeit, it is possible that there was a "basement" effect on anxiety behavior of GDX rats, such that indomethacin could not further reduce some of the behavioral measures. For example, current results in the open field and elevated plus maze indicate that GDX decreases overall locomotion. Decreased activity affects performance in these two tasks. However, indomethacin administration to GDX rats in the defensive freezing task increased motor behavior, which indicates that androgens' antianxiety effects may not be mediated through their effects to reduce activity.

Further research is needed to address the mechanisms by which 5α-reduced androgens in the hippocampus mediate anxiety behavior. Indeed, androgen receptors, which T and DHT have a high affinity for (Roselli et al., 1987), have been localized to the hippocampus (Kerr et al., 1995; Sar et al., 1990). However, 3α-diol does not typically bind with high affinity to androgen receptors (Roselli et al., 1987) in physiological concentrations. 3α-Diol has agonist-like activity at $GABA_A$ receptors (Frye et al., 1996a,b,c), which have also been localized to the hippocampus (Collinson et al., 2002). It is also possible that 3α-diol may be acting at these other substrates, such as *N*-methyl-D-aspartate receptors or via signal transduction pathways (Rhodes et al., 2004). Androgens' capacity to alter anxiety behavior may also be related to its effects on neuroplasticity. Some effects of antidepressant drugs may be related to their capacity to induce neurogenesis in the hippocampus (Santarelli et al., 2003). Recently, it has been discovered that androgens can enhance neurogenesis in the hippocampus (Leranth et al., 2003). Thus, additional research is necessary to characterize androgen's effects and determine their mechanisms of actions for anxiety behaviors.

Although the present results suggest the hippocampus may be an important site for androgen's effects, these findings do not preclude androgens having actions in other areas of the brain to mediate anxiety. For example, the nucleus accumbens, a target for 5α-reduced androgens' rewarding effects (Frye et al., 2002), may have an important role in mediating anxiety in the elevated plus maze (Martinez et al., 2002). As well, androgens may have effects to mediate fear-potentiated startle through actions in the amygdala of female rats or the bed nucleus of the stria terminalis of male rats (Walker et al., 2003). Therefore, it is necessary to consider other brain regions in addition to the hippocampus when

examining the effects of 5α-reduced metabolites on anxiety behavior. In the present study, rats with indomethacin implants to sites other than the dorsal hippocampus exhibited behavior that was similar to that of rats with control implants in the same androgen condition. Although these findings suggest that T's metabolism in the dorsal hippocampus may alter anxiety behaviors, it will also be important to further examine effects of implants to other nearby regions.

In summary, the present findings indicate that 5α-reduced metabolites can have effects in the hippocampus to mediate anxiety behaviors. First, DHT produced anti-anxiety behavior that was similar to that of intact rats, and greater than that of GDX rats. Second, indomethacin to the dorsal hippocampus of intact and DHT-replaced rats significantly increased anxiety and fear behaviors, compared to those with control implants. These data provide evidence that 5α-reduced metabolites may mediate anxiety behavior and that inhibiting formation of 3α-diol in the hippocampus can attenuate these beneficial effects of androgens on anxiety behavior.

Acknowledgements

This research was supported by grants from the Whitehall (096-010) and National Science Foundations (IBN98-96263 and IBN-0344103). We appreciate the assistance of Madeline Rhodes, Angela Seliga, Alicia Walf, and Joanna Wawryzycki in the implementation of the studies.

References

Adler A, Vescovo P, Robinson JK, Kritzer MF. Gonadectomy in adult life increases tyrosine hydroxylase immunoreactivity in the prefrontal cortex and decreases open field activity in male rats. Neuroscience 1999;89:939–54.

Aikey JL, Nyby JG, Anmuth JM, James PJ. Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). Horm Behav 2002; 42:448–60.

Amateu SK, McCarthy MM. A novel mechanism of dendritic spine plasticity involving estradiol induction of Prostaglandin-E_2. J Neurosci 2002;22:8586–96.

Bannerman DM, Deacon RM, Offen S, Friswell J, Grubb M, Rawlins JN. Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. Behav Neurosci 2002;5:884–901.

Beyer C, Gonzalez-Flores O, Ramirez-Orduna JM, Gonzalez-Mariscal G. Indomethacin inhibits lordosis induced by ring A-reduced progestins: possible role of 3α-oxoreduction in progestin-facilitated lordosis. Horm Behav 1999;35:1–8.

Bing O, Heilig M, Kakoulidis P, Sundblad C, Wikland L, Eriksson E. High doses of testosterone increase anti-conflict behavior in rat. Eur Neuropsychopharmacol 1998;8:321–3.

Bitran D, Kellog CK, Hilvers RJ. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical $GABA_A$ receptors in the rat. Horm Behav 1993;27:568–83.

Bitran D, Hilvers RJ, Frye CA, Erskine MS. Chronic anabolic-androgenic steroid treatment affects brain $GABA_A$ receptor-gated chloride ion transport. Life Sci 1996;58:573–83.

Clunie M, Crone LA, Klassen L, Yip R. Psychiatric side effects of indomethacin in parturients. Can J Anesth 2003;50:586–8.

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 2002;22:5572–80.

Dazzi L, Sanna A, Cagetti E, Concas A, Biggio G. Inhibition by the neurosteroid allopregnanalone of basal and stress-induced acetylcholine release in the brain of freely moving rats. Brain Res 1996;710: 275–80.

Dazzi L, Serra M, Vacca G, Ladu S, Latrofa A, Trapani G, et al. Depletion of cortical allopregnanalone potentiates stress-induced increase in cortical dopamine output. Brain Res 2002;932:135–9.

DeBoer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology, and psychopharmacology. J Pharmacol 2003;463:145–61.

Edinger KL, Frye CA. Testosterone's analgesic, anti-anxiety, and cognitive-enhancing effects may be due in part to actions of 5α-reduced metabolites in the hippocampus; Behav Neurosci 2004 in press.

Fernandez-Guasti A, Martinez-Mota L. Orchidectomy sensitizes male rats to the action of diazepam on burying behavior latency: role of testosterone. Pharmacol Biochem Behav 2003;75:473–9.

Frye CA, Lacey EH. Posttraining androgens' enhancement of cognitive performance is temporally distinct from androgens' increases in affective behavior. Cogn Affect Behav Neurosci 2001;2:172–82.

Frye CA, McCormick CM. Androgens are neuroprotective in the dentate gyrus of adrenalectomized female rats. Stress 2000a;3:186–94.

Frye CA, McCormick CM. The neurosteroid, 3α-androstanediol, prevents inhibitory avoidance deficits and pyknotic cells in the granule layer of the dentate gyrus induced by adrenalectomy in rats. Brain Res 2000b;855:166–70.

Frye CA, Seliga AM. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. Cogn Affect Behav Neurosci 2001;1:371–81.

Frye CA, Walf AA. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav 2002;41:306–15.

Frye CA, Walf AA. Hippocampal 3α,5α-THP may alter depressive behavior of pregnant and lactating rats; Pharmacol Biochem Behav 2004; 78;531–40.

Frye CA, McCormick CM, Coopersmith C, Erskine MS. Effects of paced and non-paced mating stimulation on plasma progesterone, 3 alpha-diol and corticosterone. Psychoneuroendocrinology 1996a;21:431–9.

Frye CA, Van Keuren KR, Erskine MS. Behavioral effects of 3α-androstanediol: I. Modulation of sexual receptivity and promotion of $GABA_A$/benzodiazepine receptors-stimulated chloride flux. Behav Brain Res 1996b;79:109–18.

Frye CA, Van Keuren KR, Rao PN, Erskine MS. Analgesic effects of the neurosteroid 3α-androstanediol. Brain Res 1996c;709:1–9.

Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex difference in performance on anxiety tests coincide with increases in hippocampal progesterone 3α, 5α-THP. Pharmacol Biochem Behav 2000;67:587–98.

Frye CA, Rhodes ME, Rosellini RA, Svare B. The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5α-reduced metabolites. Pharmacol Biochem Behav 2002;74:119–27.

Frye CA, Edinger KL, Seliga AM, Wawryzycki JM. 5α-reduced metabolites may have actions in the hippocampus to enhance cognitive performance of male rats. Psychoneuroendocrinology; 2004 [in press].

Gallo MA, Smith SS. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: a possible rat model of PMS anxiety. Pharmacol Biochem Behav 1993;46:897–904.

Isgor C, Sengelaub DR. Effects of neonatal gonadal steroids on adult CA3 pyramidal neuron dendritic morphology and spatial memory in rats. J Neurobiol 2003;5:179–90.

Ivanova T, Beyer C. Ontogenetic expression and sex differences of aromatase and estrogen-receptor alpha/beta mRNA in the mouse hippocampus. Cell Tissue Res 2000;300:231–7.

Jacobs EC, Arnold AP, Campognoni AT. Developmental regulation of the distribution of aromatase- and estrogen-receptor-mRNA-expressing cells in the zebra finch brain. Dev Neurosci 1999;21:453–72.

Kerr JE, Allore RJ, Beck SG, Handa RJ. Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. Endocrinology 1995;136:3213–21.

Leranth C, Petnehazy O, MacLusky NJ. Gonadal hormones affect synaptic density in the CA1 hippocampal subfield of male rats. J Neurosci 2003;23:1588–92.

Li X, Bertics P, Karavolas H. Regional distribution of cytosolic and particulate 5α-dihydroprogesterone 3α-hydroxysteroid oxidoreductase in female rat brain. J Steroid Biochem Mol Biol 1997;60:311–8.

MacLusky NJ, Walters MJ, Clark AS, Toran-Allerand CD. Aromatase in the cerebral cortex, hippocampus, and mid-brain: ontogeny and developmental implications. Mol Cell Neurosci 1994;5:691–8.

Martinez G, Ropero C, Funes A, Flores E, Blotta C, Londa AI, et al. Effects of selective NMDA and non-NMDA blockade in the nucleus accumbens on the elevated plus maze task. Physiol Behav 2002;76:219–24.

Mizoguchi K, Kunishita T, Chui DH, Tabira T. Stress induces neuronal death in the hippocampus of castrated rats. Neurosci Lett 1992;138: 157–60.

Oka S, Matusumoto M, Ohtake K, Kiyoshima T, Nakakimura K, Sakabe T. The addition of epinephrine to tetracaine injected intrathecally sustains an increase in glutamate concentrations in the cerebrospinal fluid and worsens neuronal injury. Anesth Analg 2001;93:1050–7.

Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.

Paxinos G, Watson C. The rat brain. New York: Academic Press; 1986.

Pelletier G, Luu-The V, Labrie F. Immunocytochemical localization of 5α-reductase in the rat brain. Mol Cell Neurosci 1994;5:394–9.

Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated-plus maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 1986;24:525–9.

Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus maze as a measure of rat anxiety. J Neurosci Methods 1984;3:149–67.

Pfaff DW. Autoradiographic localization of testosterone-^{3}H in the female rat brain and estradiol-^{3}H in the male rat brain. Experientia 1968; 24:958–9.

Pouliot WA, Handa RJ, Beck SG. Androgen modulates *N*-methyl-D-aspartate-mediated depolarization in CA1 hippocampal pyramidal cells. Synapse 1996;23:10–9.

Reilly MT, Crabbe JC, Rustay NR, Finn DA. Acute neuroactive steroid withdrawal in withdrawal seizure-prone and withdrawal seizure-resistant mice. Pharmacol Biochem Behav 2000;67:709–17.

Rhodes ME, Frye CA. Androgens in the hippocampus can alter, and be altered by, ictal activity. Pharmacol Biochem Behav 2004;78:483–93.

Rhodes ME, Harney JP, Frye CA. Gonadal, adrenal, and neuroactive steroids role in ictal activity. Brain Research 2004;1000:8–18.

Roselli CE, Horton LE, Resko JA. Time-course and steroid specificity of aromatase induction in rat hypothalamus–preoptic area. Biol Repro 1987;37:628–33.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.

Sar M, Lubahn DB, French FS, Wilson EM. Immunohistochemical localization of the androgen receptor in rat and human tissues. Endocrinology 1990;127:3180–6.

Smith MD, Jones LS, Wilson MA. Sex differences in hippocampal slice excitability: role of testosterone. Neuroscience 2002;3:517–30.

Song C, Phillips AG, Leonard B. Interleukin 1 beta enhances conditioned fear learning in rats: possible involvement of glucocorticoids. Eur J Neurosci 2003;18:1739–43.

Treit D. The inhibitory effect of diazepam on defensive burying: anxiolytic and analgesic effects. Pharmacol Biochem Behav 1984;22:47–52.

Treit D, Lolordo VM, Armstrong DE. The effects of diazepam on "fear" reactions in rats are modulated by environmental constraints on the rat's defensive repertoire. Pharmacol Biochem Behav 1986;25:561–5.

Walf AA, Frye CA. Restraint stress attenuates anti-anxiety and anti-depressive behavior produced by physiological estradiol regimen; 2004 [submitted for publication].

Walf AA, Rhodes ME, Frye CA. Antidepressant effects of ERβ-selective estrogen receptor modulators in the forced swim test; Pharmacol Biochem Behav 2004;78;523–9.

Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur J Pharmacol 2003;463:199–216.

Zimmerberg B, Brown RC. Prenatal experience and postnatal stress modulate the adult neurosteroid and catecholaminergic stress responses. Int J Dev Neurosci 1998;16:217–28.