Anxiogenic Effects of Neurosteroid Exposure: Sex Differences and Altered GABA<sub>A</sub> Receptor Pharmacology in Adult Rats

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Received October 3, 2002; accepted January 24, 2003

ABSTRACT

Acute exposure to progesterone or its neurosteroid derivative allopregnanolone (3α,5α-THP) is anxiolytic, consistent with the GABA modulatory effects of 3α,5α-THP at the GABA<sub>A</sub> receptor. However, continuous exposure to progesterone increases anxiety in association with increased expression of the benzodiazepine-insensitive GABA<sub>A</sub> receptor α4 subunit. Furthermore, negative mood symptoms and altered GABA<sub>A</sub> receptor pharmacology in patients with premenstrual dysphoric disorder occur in the early luteal phase in association with peak circulating levels of progesterone and 3α,5α-THP. Because sex differences have been reported in steroid-regulated anxiety responses, the present study investigated the role of sex and development in the regulation of anxiety after short-term exposure to 3α,5α-THP. To this end, we compared the effects of hormone administration in adult male, adult female, and juvenile female rats. Increased anxiety in the elevated plus maze was evident in all groups after 48-h exposure to either 3α,5α-THP or progesterone. At this time point, alterations in the anxiolytic profile of benzodiazepine agonists and antagonists were observed in both adult males and females in the elevated plus maze. However, sex differences in the acoustic startle response were observed after short-term hormone treatment such that only female rats displayed an increased response indicative of higher anxiety levels. These results suggest that although neurosteroid exposure may influence both the pharmacological properties of the GABA<sub>A</sub> receptor and the manifestation of anxiety in both sexes, the effects of neurosteroids may be modulated in a sex- and task-specific manner.

The regulation of anxiety is integrally associated with the function of several neurotransmitter systems, including the GABA<sub>A</sub> receptor system (Sanders and Shekhar, 1995; Sundström et al., 1997; Crestani et al., 1999; Low et al., 2000; Serra et al., 2000). There is, however, a marked difference between the acute effects of agents acting via the GABA<sub>A</sub> receptor and the effects of longer term treatment on the GABA<sub>A</sub> receptor system and on relevant behavioral outcomes. Acute treatments with several classes of drugs that positively modulate the GABA<sub>A</sub> receptor are anxiolytic (Sanders and Shekhar, 1995; Brot et al., 1997); however, chronic treatment with and/or withdrawal from these substances can have the opposite effect, leading to increased anxiety levels in association with altered expression and function of GABA<sub>A</sub> receptor (File et al., 1987; Saunders et al., 1990; Rassnick et al., 1992; Gallo and Smith, 1993; Holt et al., 1996; May et al., 1997; Smith et al., 1999a; Follesa et al., 2001, 2002). Endogenous modulators of the GABA<sub>A</sub> receptor, such as the neuroactive metabolites of steroid hormones, are also known to affect GABA<sub>A</sub> receptor function and expression. Therefore, the regulation of GABA<sub>A</sub> receptor expression and function by neurosteroids may be essential for understanding the etiology and treatment of anxiety.

The GABA<sub>A</sub> receptor is a ligand-gated chloride channel, the functional properties of which depend on its subunit composition (Wafford et al., 1996). Potentiation of the GABA<sub>A</sub> receptor by several of its modulators, including benzodiazepines and the benzodiazepine antagonist flumazenil (FLU), is dependent on the isoform of the receptor. Benzodiazepines, such as lorazepam, for example, are generally positive modulators of GABA-gated current when the GABA<sub>A</sub> receptor contains a γ-subunit in combination with α1–3 or 5 (Wafford et al., 1996). However, GABA<sub>A</sub> receptors containing α4 subunits are insensitive to lorazepam and are instead positively modulated by FLU (a.k.a. RO 15-1788) (Wafford et al., 1996).

The neurosteroid allopregnanolone (3α,5α-THP) is also potent positive modulator of GABA-gated current and, in common with several other classes of drugs that act via the GABA<sub>A</sub> receptor, is anxiolytic when acutely applied (Bitran et al., 1993; Akwa et al., 1999). In contrast, prolonged exposure to this neurosteroid produces time-dependent anxiogenic effects. Continuous exposure to progesterone or 3α,5α-THP for 48 to 72 h increases anxiety in association with...
insensitivity to benzodiazepine agonists and increased expression of the α4 GABAAR receptor subunit (Gulinello et al., 2001). However, by 5 days of prolonged steroid exposure, anxiety responses, α4 levels, and benzodiazepine pharmacology have returned to control values and remain unaltered during continuous steroid exposure until steroid “withdrawal” (Smith et al., 1998a; Gulinello et al., 2001) at which time these parameters are again altered 8 to 48 h after cessation of steroid administration. Benzodiazepine insensitivity associated with chronic, short-term 3α,5α-THP treatment has also been replicated in cell culture (Friedman et al., 1993; Yu et al., 1996). In addition, chronic treatment with other GABA modulatory agents also increases expression of the α4 subunit (Holt et al., 1996; Ramsey-Williams and Carter, 1996; Devaud et al., 1998), although the time course of these effects is not identical between different classes of drugs, and other groups have reported divergent findings that may be a function of brain region, dosing paradigm, gender, and cell line in addition to other factors (Devaud et al., 1998; Grobin et al., 2000; Arnott et al., 2001; Follesa et al., 2001, 2002).

A change in anxiety state in association with neurosteroid exposure may be pertinent to patients with premenstrual mood disorders, who also demonstrate increased anxiety and insensitivity to benzodiazepines in association with peak levels of progesterone in the luteal phase (Wang et al., 1996; Sundström et al., 1997). Furthermore, several studies demonstrate that 3α,5α-THP may be a relevant modulator of both GABAAR receptor subunit expression and behavior in males as well as females (Corpechot et al., 1993; Steimer et al., 1997; Concasa et al., 1998, 1999; Gomez et al., 1998; Serra et al., 2000; Gulinello et al., 2002). Levels of 3α,5α-THP are also correlated with symptoms of mood disorders in males (Uzunova et al., 1998; Strohle et al., 1999). These data are corroborated by animal models of mood disorders that also demonstrate altered levels of 3α,5α-THP in conjunction with changes in GABAAR receptor subunit expression and altered GABAAR receptor pharmacology in males (Drugan et al., 1989; Park et al., 1993; Steimer et al., 1997; Serra et al., 2000).

Therefore, although there is evidence to suggest that 3α-5α-THP levels may be involved in the regulation of GABAAR receptor subunit expression and relevant behavioral outcomes, there are, however, some issues that remain to be clarified. It is a matter of some controversy whether increases or decreases in the levels of 3α-5α-THP are associated with mood disorders in humans. Symptoms of depression and anxiety are associated with decreased levels of 3α-5α-THP in major unipolar depression and in rodent models of depression and anxiety (Romeo, 1998; Uzunova et al., 1998; Guidotti et al., 2001), whereas, in contrast, increased levels of 3α-5α-THP are associated with anxiety disorders and negative mood symptoms in premenstrual dysphoric disorder (Wang et al., 1996). Some of these differences may reflect the differing modes of action of acute increases in neurosteroids, which are generally anxiolytic (Bitran et al., 1993; Brot et al., 1997; Frye and Walf, 2002) and have a negative feedback effect on the stress responses (Drugan et al., 1994; Patchev et al., 1994; Guo et al., 1995), and longer term exposures, which can regulate atypical GABAAR receptor subunit levels, thus resulting in altered GABAAR receptor kinetics and GABAergic transmission (Smith et al., 1998a,b; Gulinello et al., 2002). It has also been suggested that the effects of neurosteroid exposure may be sex-dependent (Wilson and Biscardi, 1997; Fernández-Guasti and Picazo, 1999; Zimmerberg et al., 1999), which may further complicate attempts to elucidate the role of neurosteroids in affective syndromes.

We therefore investigated the effects of short-term neurosteroid exposure on anxiety and the predicted changes in the anxiolytic profile of GABAAR receptor modulators in the elevated plus maze. To determine whether the effects of 3α-5α-THP and its parent compound, progesterone, are dependent on ovarian status or sex, we used adult male rats, adult female rats, and juvenile female rats. Because the effects of several anxiolytic and anxiogenic agents can be task-dependent (Johnston and File, 1991), we also assessed anxiety levels in the acoustic startle response (ASR). The ASR is a whole body response to acoustic stimuli that has a similar circuitry and pharmacology in humans (Koch, 1999). Altered ASR has been demonstrated in anxiety and depressive disorders in humans (Allen et al., 1999) and in animal models of these disorders (Schwegler et al., 1997).

**Materials and Methods**

**Animals**

Adult male, adult female, and juvenile female Long-Evans rats (Charles River Laboratories, Raleigh, NC) were housed in single-sex pairs in the same room under a 14-h light and 10-h dark cycle with food and water ad libitum. Adult rats were 2–3 months old (200 ± 25 g) and juvenile female rats were 23 to 25 days old (60–75 g, after weaning but before puberty) at the start of each experiment. All animals were tested during the light portion of the circadian cycle between 9:00 AM and 2:00 PM. In adult female rats, estrous cycle stage was determined by microscopic examination of the vaginal lavage and by measures of vaginal impedance, as described previously (Gulinello et al., 2001). Male rats and juvenile female rats were handled for the same amount of time. Animals were randomly assigned to hormone and treatment groups, and animals not in diestrous were excluded from the experiments, which eventually resulted in unequal numbers of subjects per group in some experiments. All animal care was conducted in accordance with guidelines provided by the Institutional Animal Care and Use Committee.

**Drugs and Hormone Administration**

Animals were injected (intraperitoneally) with either progesterone (P, 5 mg/kg in sesame oil), 3α,5α-THP (3α-OH-5α-pregnan-20-one, 10 mg/kg in sesame oil) once each morning (between 9:00 AM and 10:00 AM) over a 48-h period for a total of three injections during this period. Injection volumes were 0.250 ml/adult animal and 0.060 to 0.075 ml/juvenile animal (depending on body weight). These doses of progesterone and 3α,5α-THP result in hippocampal levels of the neurosteroid, which are physiological (6–7 ng/g) (Moran and Smith, 1998; Frye and Bayon, 1999). Control animals were given the same number of injections of vehicle (sesame oil).

Animals were tested 3 to 4 h after the final hormone injection. On the day of testing, animals were injected intraperitoneally with flumazenil (10 mg/kg) or lorazepam (0.75 mg/kg) 12 to 15 min before testing with FLJ or 45 min before testing with lorazepam. Control animals were injected similarly with vehicle (1.8% polyethylene glycol 400 in propylene glycol with 4 drops of Tween 80). Due to developmental differences in GABAAR receptor pharmacology and subunit expression between prepubertal and adult animals, only adult animals were used to test the pharmacological profile of lorazepam and flumazenil (Barr and Lithgow, 1983; Araki et al., 1996). Chemicals were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated. Lorazepam (injectable) was obtained from Wyeth-Ayerst (Princeton, NJ) and flumazenil was from Tocris Cookson Inc. (Ballwin, MO).
Behavioral Testing

Elevated Plus Maze. Rats were tested on the plus maze, elevated 50 cm above the floor, in a room with low, indirect lighting and low noise levels. The plus maze consists of two enclosed arms (50 × 10 × 40 cm) and two open arms (50 × 10 cm). The apparatus was thoroughly cleaned with 70% ethanol after each trial. The open arms had a small rail outside the first half of the open arm as described previously (Gulinello et al., 2001). The floor of all four arms was marked with grid lines every 25 cm. On the day of testing each rat was transferred to the testing room and acclimatized for 1 h before testing, and then placed in a start box in the center of the plus maze and tested for 10 min after exiting the start box into the plus maze. To be considered as an entry into any arm, the rat must pass the line of the open platform with all four paws. The duration (in seconds) of time spent in the open arm was recorded from the time of entry into the open arm. Decreased time spent in the open arm generally indicates higher levels of anxiety because rodents have an intrinsic preference for closed rather than open elevated spaces (Handley and Mithani, 1984; Pellow and File, 1986; Cruz et al., 1994). To measure general locomotor activity, the number of total grid crosses was counted. The percentage of open arm entries compared with total entries is a further measure of anxiety-like behavior indicated under results as percentage of open arm entries (Pellow et al., 1985). Data from adult males and females in the elevated plus maze were first analyzed in a two-way ANOVA (hormone condition × sex) followed by a post hoc Fisher’s PLSD t test. Data from juvenile females were analyzed separately in a one-way ANOVA (hormone condition) followed by a post hoc Fisher’s PLSD t test. Data from each graph represent a separate group of animals tested in either the elevated plus maze or the acoustic startle test because multiple trials in the elevated plus maze do not reliably result in the same pattern of responses (File et al., 1993; Bertoglio and Carobrez, 2002) and prior exposure to stressful experiences can also influence performance on subsequent tests of emotional behavior (DaCunha et al., 1992; Andrews and File, 1993; Bertoglio and Carobrez, 2002).

Acoustic Startle. Because of the gross differences in body weights and in the development of the ASR between adult and juvenile animals, juvenile animals were not tested in the startle paradigm (Gallager et al., 1983). Acoustic startle magnitude (Fleshler, 1965; Szabo, 1967) was assessed using an S-R Lab Apparatus (San Diego Instruments, San Diego, CA). Rats were placed in a 20 × 32-cm Plexiglas cylinder attached to a piezoelectric transducer platform to detect the motion of the rat. Movement of the platform results in a voltage change in the transducer that was digitized and analyzed by the S-R Lab program on an attached computer. After a 5-min period of acclimatization to 65-dB background noise, rats were presented with 10 consecutive trials of 120-dB sound pulses of 40-ms duration in a habituation trial. Immediately thereafter, startle magnitude and threshold were assessed by presentation of broadband noise of varying intensities (0, 90, 110, or 120 dB) a total of five times in random order with random time intervals separating each trial. Startle magnitude was defined as an average of responses to each stimulus intensity and is illustrated under results as the maximum startle response. Habituation trials were performed for several reasons. This protocol results in reliable responses to the stimuli, whereas the responses to the first several presentations are more variable (Hoffman and Stitt, 1969; Schwarzkopf et al., 1993). Some groups have indicated that habituation and acclimatization periods are important factors in sex comparisons (Schwarzkopf et al., 1993; Lehmann et al., 1999; Faraday and Grunberg, 2000).

Results

Forty-Eight Hour Hormone Treatment Increases Anxiety in the Elevated Plus Maze in Male, Female, and Juvenile Female Rats. Administration of either progesterone or 3α,5α-THP for 48 h significantly increased anxiety in adult rats of both sexes and in juvenile female rats. Both 48-h P and 48-h neurosteroid treatment (3α,5α-THP) significantly decreased the time spent in the open arm by roughly 2-fold in comparison with vehicle-injected controls (Fig. 1; p < 0.01 for male, female, and juvenile females). Exposure to 3α,5α-THP or progesterone also decreased the absolute number of open arm entries (Fig. 1) and the percentage of open arm entries (Fig. 1) by roughly 50%. There were no significant effects of sex across treatment conditions in any plus maze measures. There were no significant differences in locomotor activity across treatment conditions as measured by total number of grid crosses (Fig. 1), number of closed arm entries, and total number of entries. Treatment with 3α,5α-THP was not significantly different from progesterone in any plus maze parameter.

Data from the elevated plus maze were first analyzed in a two-way ANOVA (hormone condition × sex, dfcondition = 2, dffsex = 2, dfcondition × sex = 2, dfresidual = 54) for adult animals

![Fig. 1. Forty-eight-hour exposure to progesterone or 3α,5α-THP increases anxiety in the elevated plus maze. A to C, anxiety measures. Forty-eight-hour exposure to P (5 mg/kg) or 3α,5α-THP (10 mg/kg) significantly increased anxiety in female (open columns), male (closed columns), and juvenile female (gray columns) rats. For this and the following graphs, sample size is indicated at the base of the columns in D, and significant effects relative to controls at p < 0.01 is indicated by *, A, anxiety-like behavior assessed as time spent in the open arm (in seconds), B, absolute number of open arm entries, C, number of open arm entries (OA entries) corrected for locomotor activity by presenting these data as a percentage of total arm entries (% OA entries). D and E, locomotor activity. General locomotor activity is illustrated as total number of grid crosses in a 10-min test period in D. There were no significant effects of locomotor activity across treatment conditions in adult male or female rats. 3α,5α-THP significantly decreased locomotor activity in juvenile female rats. Steroid administration did not alter the number of closed arm entries (E, CA entries).]

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(males and females). There were significant effects of hormone condition on time spent in the open arm (F = 52.847, p < 0.001); however, there were no significant effects of sex (p = 0.2436) and no interaction of hormone condition and sex (p = 0.8081) on time spent in the open arm. A post hoc t test was then performed. Adult control animals of both sexes spent significantly more time in the open arm than adult animals injected with either progesterone (female and male, Fisher’s PLSD, p < 0.001) or 3α-5α-THP (female and male, Fisher’s PLSD, p < 0.001). Juvenile female control animals (F = 19.540, df_condition = 2, df_residual = 24, p < 0.001) similarly spent more time in the open arm than either progesterone- (Fisher’s PLSD, p < 0.001) or 3α-5α-THP (Fisher’s PLSD, p < 0.001)-injected juvenile females. The number of open arm entries (F = 30.519, p < 0.001) and the percentage of open arm entries (F = 19.540, p < 0.001) were similarly affected by 48-h steroid exposure. Adult animals of either sex injected with vehicle exhibited a significantly higher number and percentage of open arm entries than animals injected with either progesterone or 3α-5α-THP (p < 0.002 for all conditions in both males and females). In contrast, neither grid crossings (F = 2.755, p < 0.7) or closed arm entries (F = 1.724, p < 0.19) was significantly altered by hormone treatments.

Forty-Eight Hour Progesterone Treatment Alters the Anxiolytic Effects of Flumazenil and Lorazepam in the Elevated Plus Maze. Administration of progesterone significantly altered the anxiolytic effects of lorazepam and flumazenil (ANOVA hormone condition × sex × drug condition; df_hormone condition = 1, df_drug condition = 2, df_gender = 2, df_residual = 86; F_hormone condition = 35.412, p < 0.001; F_drug condition = 18.717, p < 0.001). There were no significant sex differences across treatment groups as indicated by a lack of significant interactions of sex with hormone condition (F = 0.35, p < 0.85) or drug condition (F = 1.853, p < 0.16). In contrast, there were significant interactions between progesterone treatment and drug condition (F = 59.934, p < 0.001). Injections of lorazepam (LZM) after 48-h administration of progesterone (P/LZM) were significantly less anxiolytic than lorazepam injections in control rats (Fig. 2). Injections of lorazepam after progesterone exposure decreased the time spent in the open arm, the number of open arm entries, and the percentage of open arm by approximately 50% relative to lorazepam injections in control rats of both sexes (LZM versus P/LZM, p < 0.01; Fig. 2).

In contrast, FLU was without effect in control animals but it became significantly anxiolytic in progesterone-injected animals of both sexes (FLU versus P/FLU, p < 0.01; Fig. 2). FLU injections after progesterone exposure increased the time spent in the open arm, the number of open arm entries, and the percentage of open arm entries by 50 to 60%. This alteration of the anxiolytic effect of flumazenil and lorazepam is consistent with up-regulation of the α4 subunit of the GABA_A receptor, because α4-containing GABA_A receptor isoforms are insensitive to modulation by lorazepam and are instead positively modulated by flumazenil.

Forty-Eight Hour Hormone Treatment Increases the Acoustic Startle Response in Female Rats. Exposure to elevated neurosteroid levels after 48-h administration of P, and after 48-h administration of 3α-5α-THP significantly increased the peak acoustic startle response (Fig. 3; ANOVA, F = 6.921, p < 0.002, df_condition = 2, df_residual = 102). Exposure to either progesterone (p < 0.001) or 3α-5α-THP (p < 0.007) in female rats increased the peak ASR 2- to 3-fold over vehicle-injected controls. There was no significant difference in peak ASR between 3α-5α-THP- or progesterone-treated groups. In contrast to the increased ASR demonstrated by female rats after hormone exposure, male rats exposed to progesterone did not demonstrate an altered ASR relative to vehicle-injected controls (Fig. 3).

Discussion

These data demonstrate that short-term exposure to the neurosteroid 3α-5α-THP increases anxiety and alters the anxiolytic potential of the benzodiazepine ligands lorazepam and flumazenil. This pharmacological profile is highly indicative of increased expression of the GABA_A receptor α4 subunit. Elevated anxiety levels after 48-h administration of
Fig. 3. Administration of progesterone or 3α,5α-THP increases the acoustic startle response in female rats. A, higher levels of anxiety and arousal were indicated by an elevated peak startle response (ASR peak, y-axis) to varying intensities of acoustic stimuli (x-axis) in female rats after administration of progesterone (P Fem, n = 9 closed circles) or 3α,5α-THP (THP Fem, n = 7, shaded circles) relative to vehicle-injected controls (n = 11, open circles). Significant differences between P-treated and control groups are indicated by * and between 3α,5α-THP and controls indicated by + at p < 0.02. B, in contrast to the increased ASR demonstrated in female rats after hormone treatments, the peak ASR was not significantly different in male hormone-treated rats (P male, dark triangles, n = 6) relative to vehicle-injected controls (control male, open triangles, n = 6).

progesterone or 3α,5α-THP were evident in both sexes when anxiety was assessed in elevated plus maze and in the acoustic startle paradigm in female rats.

It is likely that the role of progesterone in the regulation of anxiety is mediated via 3α,5α-THP. These data demonstrate that anxiogenic effects of progesterone exposure are replicated by direct exposure to 3α,5α-THP in female rats in two separate anxiety measures: the elevated plus maze and the acoustic startle paradigm. Furthermore, previous studies indicate that inhibition of neurosteroid synthesis during progesterone exposure prevents the up-regulation of the GABA \(_\text{A}\) receptor \(\alpha_4\) subunit and changes in anxiety levels (Smith et al., 1998a,b; Follesa et al., 2000; Frye et al., 2000). However, we cannot rule out the possibility that prolonged exposure to progesterone also alters GABAergic transmission and/or anxiety-like behavior via its classical actions on gene transcription.

Although acute alterations in brain 3α,5α-THP concentrations can alter anxiety (Bitran et al., 1993; Frye et al., 2000), it is unlikely that the alterations in anxiety levels evident here are a function of the 3α,5α-THP concentration at the time of testing. First, animals were tested several hours after the final treatment with 3α,5α-THP or progesterone. Second, even if total 3α,5α-THP levels were not substantially decreased at the time of testing, high levels of 3α,5α-THP are generally anxiolytic (Bitran et al., 1993; Akwa et al., 1999; Frye et al., 2000). Therefore, if endogenous levels of 3α,5α-THP at the time of testing were the major factor regulating anxiety at the time of these tests, one would expect progesterone- and 3α,5α-THP-injected rats to be significantly less anxious than controls, which was not the case. In contrast, we suggest that changes in GABA \(_\text{A}\) receptor expression and function due to hormone exposure, may underlie the increased anxiety evident after 48-h exposure to elevated 3α,5α-THP levels.

We have previously demonstrated that treatment with either progesterone or 3α,5α-THP for 48 h increases hippocampal expression of the \(\alpha_4\) subunit of the GABA \(_\text{A}\) receptor (Gulinello et al., 2001). The \(\alpha_4\)-containing GABA \(_\text{A}\) receptors have a distinctive pharmacology such that they are insensitive to the modulatory effects of benzodiazepine agonists such as lorazepam, but are instead positively modulated by the benzodiazepine antagonist flumazenil. The increase in functional \(\alpha_4\)-containing GABA \(_\text{A}\) receptor was confirmed here at a behavioral level by a comparative insensitivity to anxiolytic effects of the benzodiazepine agonist lorazepam and agonist-like properties of the benzodiazepine antagonist flumazenil after 48-h treatment with progesterone in both male and female rats in the elevated plus maze.

We have previously published similar results obtained after administration of progesterone via a subcutaneously implanted progesterone capsule (Gulinello et al., 2001). These data would argue against the possibility that the injection protocol used in the present study results in a short withdrawal paradigm. For progesterone implants, steroid levels remain at high, steady-state concentrations during the anxiety testing procedure at which time we have previously demonstrated similarly altered anxiety levels and benzodiazepine responses as are reported in the present study. Our previous studies (Smith et al., 1998a,b) also demonstrate that the effects of steroid withdrawal do not occur until 8 h after termination of progesterone treatment, and animals in the present study were tested 3 to 4 h after the last steroid injection. Therefore, it is unlikely that the altered anxiety-like behavior that we have demonstrated here is solely the result of cessation of hormone treatment, but rather due to continuous exposure to neurosteroids. Furthermore, similar pharmacological changes occur after 48-h neurosteroid exposure in cell cultures (Friedman et al., 1993).

Several other studies have also demonstrated changes in GABA \(_\text{A}\) receptor function and expression after exposure to 3α,5α-THP (Bitran et al., 1991; Finn and Gee, 1993; Friedman et al., 1993; Yu et al., 1996; Belmar et al., 1998). Furthermore, altered anxiolytic effects of GABA \(_\text{A}\) receptor modulators have also been demonstrated after these relatively short-term exposures to neurosteroids (Bitran et al., 1991; Fernandez-Guasti and Picaoz, 1997). In fact, GABA \(_\text{A}\) receptor subunit switching may also occur very rapidly during exposure to progesterone (Weiland and Orchinik, 1995; Brussaard et al., 1997) or after exposure to stressors that substantially increase brain 3α,5α-THP concentrations (Orchinik et al., 1995; Barbaccia et al., 1996). Taken together, these data suggest that the manifestation of anxiety-like behavior and the altered modulatory effects of GABA \(_\text{A}\) receptor ligands may be regulated by the common mechanism of subunit-selective expression.

There is ample evidence that acute secretion of progesterone and neurosteroids are protective against the damaging
effects of stressors (Drugan et al., 1994; Guo et al., 1995; Putchek and Almeida, 1996), and this may be one mechanisms by which altered neurosteroid levels could occur in males (Barbaccia et al., 1996; Serra et al., 2000). However, prolonged exposure to 3α,5α-THP may dysregulate these responses and render the GABA_α receptor insensitive to neurosteroids, and may thus predispose subjects to negative effects of stressors (Drugan et al., 1994; Serra et al., 2000).

In fact, several studies have linked negative mood symptoms in both sexes to alterations in neuroactive steroid levels in association with altered GABA_α receptor function (Sundström et al., 1997; Schmidt et al., 1998; Uzunova et al., 1998; Serra et al., 2000). These data suggest that manifestation of negative mood symptoms may be correlated with alterations of GABA_α receptor subunit expression during exposure to neurosteroids.

There are, however, indications that females may be more susceptible to modulation of anxiety-like behavior by neurosteroids or that these effects may be more widespread in females. These data are important in light of the fact that there are notable sex differences in the prevalence of mood disorders in humans (Kessler et al., 1994; Pigott, 1999). Although there were no sex differences in anxiety-like behavior, or in the pharmacological effects of GABA_α receptor modulators in the elevated plus maze after hormone treatments, there were sex differences in the ASR after hormone exposure. Females startle significantly more after 48-h exposure to either progesterone or 3α,5α-THP, whereas males do not.

It has elsewhere been demonstrated that GABA_α receptor expression is regulated by exposure to GABA_α receptor modulators in a sex-specific manner in specific brain regions important in the regulation of the ASR, such as the amygdala (Papadeas et al., 2001). There are also notable sex differences in the hormonal regulation of several other major neurotransmitter systems that also regulate the ASR, including the glutamatergic system (Cyr et al., 2000) and the serotonergic system (Maswood et al., 1999; Zhang et al., 1999). Recent evidence also suggests that continuous neurosteroid exposure in females also regulates neuropeptide expression in the amygdala (Ferrara et al., 2001). Therefore, several other factors may also contribute to the sex differences observed here and in sexually dimorphic responses to stressors in general (Akinici and Johnston, 1993; Figueiredo et al., 2002).

These factors may include other steroid hormones in addition to progesterone. In contrast to progesterone exposure, chronic exposure to testosterone decreases anxiety and increases GABA-stimulated chloride flux (Bitran et al., 1996). Furthermore, the higher estrogen levels in females may also be important in the manifestation of anxiety after progesterone exposure in females (Shors et al., 1999), because estrogen can interact with progesterone and 3α,5α-THP to regulate neuronal excitability and anxiety (Cyr et al., 2000; Laconi et al., 2001) and may also have independent effects on synaptic transmission (Woolley and McEwen, 1994; Cyr et al., 2000). These data suggest that both the acute and organizational effects of hormones may be involved in the divergent responses of males and females to neurosteroid exposure in specific tasks, via differential actions in different brain regions and/or neurotransmitter systems.

The results from the present study demonstrate a change in behavior in two widely used animal models of anxiety, the elevated plus maze and the acoustic startle response. Increases in “anxiety-like” behavior have been demonstrated in both tasks after withdrawal from progesterone (Gulineillo et al., 2002, 2003), when responses to GABA-modulators is altered (Sundström-Poromaa et al., 2002). More globally increased anxiety assessed by these tasks is also seen after withdrawal from GABA-modulatory drugs (Ryan and Boisse, 1983; File et al., 1987; Rassnick et al., 1992; Moy et al., 1997).

In fact, the ASR may be increased in subjects suffering from post-traumatic stress disorders (Morgan et al., 1996; Shalev et al., 2000) and during anticipatory anxiety (Grillon et al., 1991) and in several classes of anxiety disorders (Jety et al., 2001; Kumari et al., 2001). Use of both tests, as in the present study, provides a more complete analysis of the behavioral state to both proximal and distal threats produced by 48-h steroid exposure (Rodgers, 1997).

In summary, these data indicate that relatively short exposures to elevated neurosteroid concentrations can result in increased anxiety. Furthermore, neurosteroids can alter the anxiolytic effects of several GABA_α receptor modulators, which strongly indicates altered GABA_α receptor subunit expression. These phenomena occur in both sexes, suggesting that neurosteroid regulation of GABA_α receptor expression may be relevant not only to premenstrual mood symptoms but also to affective disorders in males. The anxiogenic effects of short-term neurosteroid exposure also seem to be more widespread in females, which may have implications for the observed sex differences in the prevalence of human mood disorders.

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