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Modulation of the discriminative stimulus effects of triazolam across the menstrual cycle phase in healthy pre-menopausal women

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Abstract

Preclinical studies indicate that changes in progesterone levels across menstrual cycle phases modulate the behavioral effects of sedative drugs acting at GABA_A receptor sites. In this study, seven healthy women learned to discriminate triazolam (0.25 mg/70 kg) from placebo. After acquiring the discrimination, a range of triazolam doses (0.00, 0.06, 0.12 and 0.25 mg/70 kg) was tested during the early follicular and mid-luteal menstrual cycle phases. During the mid-luteal phase, when progesterone levels were elevated, 0.12 mg/70 kg triazolam was identified as the active triazolam training dose (0.25 mg/70 kg), whereas 0.12 mg/70 kg triazolam was identified as placebo during the early follicular phase, when progesterone levels were low. Triazolam engendered prototypical sedative effects on subjective effect, performance and cardiovascular measures that were generally independent of cycle phase. These results suggest that the discriminative stimulus effects of the positive GABA_A modulator, triazolam, are sensitive to menstrual cycle phase in healthy adult women.

Keywords

Menstrual Cycle; Drug Discrimination; Progesterone; Triazolam; Human

1. Introduction

Progesterone is a positive modulator of the GABA_A receptor complex, and its primary metabolites allopregnanolone and TH-DOC function as ligands at extracellular steroid-specific recognition sites on GABA_A receptors with affinities that are comparable to those of many benzodiazepines (Bertz et al., 1995; Paul & Purdy, 1992; Majewska, 1992; Majewska et al., 1986). Through GABA_A modulation, progesterone induces behavioral effects similar to those engendered by benzodiazepines (e.g., anxiolysis, sedation and antiepileptic effects) (Frye, 2007; Rupprecht, 2003; Schumacher et al., 1989), and changes in progesterone levels across the menstrual cycle have been shown to modulate the behavioral effects of GABAergic drugs in preclinical models. Grant and colleagues, for example, demonstrated that sensitivity to the discriminative-stimulus effects of alcohol was enhanced in non-human primates during the

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mid-luteal phase of the menstrual cycle when progesterone levels are elevated compared to the follicular phase when progesterone levels are minimal (Grant et al., 1997, Green et al., 1999). In contrast, few changes in the reinforcing or subjective effects of ethanol or triazolam have been reported across the menstrual cycle in normally cycling, healthy women (Holdstock & de Wit, 2000; Rukstalis & de Wit, 1999). Inconsistencies in menstrual cycle phase modulation of sedative drug effects across studies could be related to differences in either species or methods used to assess drug effects. The current study addressed these inconsistencies by examining the discriminative stimulus effects of triazolam during the mid-luteal and early follicular phases of the menstrual cycle in normally cycling, healthy women. It was hypothesized that the discriminative stimulus effects of triazolam would be enhanced during the mid-luteal phase.

2. Method

2.1 Participants

Healthy pre-menopausal women were recruited through local newspaper advertisements and with flyers posted on a university campus. All potential participants completed an initial telephone or internet-based screening questionnaire, and an on-site medical evaluation that included a menstrual history, health questionnaires, blood chemistry and urinalysis. Eligibility criteria included a minimum of 18 years of age, good health, reports of intermittent sedative drug use (e.g., alcohol) and regular menstrual cycles (monitored across two menstrual cycle phases prior to participation). Exclusion criteria included significant medical history (e.g., cardiovascular, neurological, or major psychiatric illnesses, including drug or alcohol dependence), regular use of drugs or alcohol, other than nicotine or caffeine, use of hormonally-based contraceptives, pregnant or breastfeeding status, or any other condition that for which triazolam administration would be contraindicated.

Twenty participants met eligibility criteria and initiated the study. Eight participants withdrew from the study for reasons unrelated to the study protocol, five were discontinued from the study because they did not acquire the drug discrimination; data from these thirteen participants were excluded from analyses. Seven volunteers completed the study. The participants ranged in age from 21 to 43 years (mean = 30 years), in weight from 47 to 69 kg (mean = 60 kg), and drank 1 to 6 alcohol drinks per month (mean = 2). One participant was a smoker (mean of 10 cigarettes per day); however, she abstained from smoking for the duration of each session.

2.2 Design

A double-blind, placebo-controlled, randomized within-subject design was used to assess the effects of menstrual cycle phase (early follicular and mid luteal), triazolam dose (training doses: 0.0, and 0.25 mg/70 kg, test doses: 0.06, 0.12 mg/70 kg), and time (assessments occurring pre-dose, 30, 60, 90, 120 and 150 minutes post-dose) on the discriminative stimulus and other behavioral effects of triazolam. Upon arrival at the laboratory, participants provided urine and breath samples that were screened for recent drug (OnTrack Teststik Bar, Varian, Inc., Lake Forest, CA) and alcohol use (Alco-Sensor III, Intoximeters, Inc., St. Louis, MO) and pregnancy (Clearview HCG II, Unipath, Ltd., Inverness Medical North America, Princeton, NJ). Assessments used to measure drug effects included a Drug Discrimination Task (post-dose only), Visual Analog (VAS) and Adjective Rating (ARS) Scales, Digit Symbol Substitution Task (DSST), and heart rate and blood pressure measurement (Sentry II, NBS Medical, Costa Mesa, CA). Descriptions of assessment measures have been provided elsewhere (e.g., Lile et al., 2007), and only the Drug Discrimination Task (Kelly et al., 1997; Oliveto et al., 1992, 1995) will be described here.

2.3 Drug-Discrimination Task

During this task, two circles labeled with a color code (i.e., “Red” and “Blue”) were displayed on the computer screen, each associated with a training dose condition. Counters were displayed directly below the circles. A mouse controlled the location of a cursor on the screen, and mouse button presses with a cursor positioned on a circle increased the counter associated with that circle according to a fixed-interval 1-sec schedule. The cursor could alternate across the circles without any consequence under the fixed-interval schedule (i.e., no change-over-delay). Up to 60 points could be allocated across the two options. The dependent variable for this task was the percent responding on the active drug-appropriate option.

2.4 Drug discrimination procedure

Sampling Phase—The sampling phase consisted of four sessions; each of the triazolam training doses (0.00 and 0.25 mg/70 kg) was administered on two sessions in an alternating pattern. For every participant, the training doses were associated with a fixed color code throughout the study, with the color-dose combinations and order of presentation being randomized across participants. Dose color was identified during drug administration, and participants were told to associate the effects of the dose with that color. Sampling sessions were conducted without regard to menstrual cycle phase.

Control Phase—Training doses of triazolam (0.00, 0.25 mg/70 kg) were administered randomly under double-blind conditions, and participants were not told which dose was administered until after the last assessment of a session. Acquisition of drug discrimination was established when $\geq 80\%$ of responses were allocated to the drug-appropriate stimulus during the last assessment of the session (150 minutes post-dose) for 5 consecutive sessions. If the acquisition criterion was not met within 15 sessions, participation ended. Participants who met criteria did so within 5 to 15 sessions. Control sessions were conducted without regard to menstrual cycle phase.

Test Phase—Test Phase sessions were identical to control sessions, except that both training and test (0.06 and 0.12 mg/70 kg) doses were presented, and sessions were scheduled only during specific phases of the menstrual cycle. Participants completed 6 sessions during the early follicular phase of the menstrual cycle (days 1-7 following onset of menses) and 6 sessions during the mid-luteal phase of their menstrual cycle (days 5-12 following ovulation, as determined using daily urine assessments). During each cycle phase, each training dose was administered once and each test dose was administered twice. The order of menstrual cycle phase testing (i.e., whether test sessions began during the early follicular or mid-luteal phase) varied among participants. Sessions were scheduled around participants' availability within each menstrual cycle phase. Because time during any particular cycle phase was limited, participants completed the Test Phase across two or three consecutive cycle phases (i.e., across several months), such that some sessions were consecutive while others were separated by days or weeks.

Task Earnings—After the final assessment on every session, participants were told whether a red dose, a blue dose, or a test dose had been administered. On red and blue dose (i.e., training) sessions, participants received \$0.04 per point accumulated on the circle associated with the dose administered on that session. On test dose sessions, participants received the average of earnings on training dose sessions, regardless of point distributions.

2.5 Data Analysis

Triazolam effects were analyzed using repeated-measures ANOVA with triazolam dose, menstrual cycle phase and time as factors. Interactions were evaluated using simple-effects analyses.

3. Results

3.1 Drug Discrimination Task

Figure 1 presents mean drug-appropriate responding as a function of triazolam dose during control and test sessions. During the final 5 control sessions, which occurred at different menstrual cycle phases across participants, drug-appropriate responding emerged only after the active dose of triazolam was administered [dose by time interaction: $F(4, 24) = 14.78, p < .001$], and greater than 80% responding occurred reliably on the drug-appropriate option for all participants beginning at 90 minutes post dose. During the test phase, active drug-appropriate responding varied as a function of menstrual cycle phase [dose $\times$ phase interaction: $F(12, 72) = 2.13, p = .025$], with simple effects analysis indicating significant cycle phase effects occurring at the 0.12 mg/70 kg test dose [$F(9, 27) = 7.90, p < .001$]. During the early follicular phase, three of seven participants identified placebo as triazolam, resulting in intermediate levels of responding when averaged across all participants, but responding was not significantly different from that occurring during the mid-luteal phase at this dose. The 0.06 mg/70 kg dose was identified as placebo during both cycle phases. The 0.12 mg/70 kg test dose was identified as triazolam during the mid-luteal phase, whereas during the early follicular phase, responding was comparable to that observed following placebo administration. The active training dose of triazolam (0.25 mg/70 kg) engendered triazolam-appropriate responding during both menstrual cycle phases.

3.2 Adjective Rating Scale

A dose by menstrual cycle phase interaction was detected on the Stimulant subscale of the ARS [$F(3, 372) = 2.67, p = .047$], with ratings at the 0.06 mg/70 kg dose showing greater decreases during the mid-luteal phase relative to the early follicular phase [simple effects: $F(1, 18) = 4.7, p = .044$].

3.3 Other Measures

Consistent with previous studies (e.g., Rush et al., 2000; 1999; Stoops et al., 2005), triazolam engendered prototypical sedative effects on many self-report, performance, and cardiovascular measures (Table 1). Baseline cardiovascular measures varied across cycle phases (e.g., Ounis-Skali et al., 2006), but triazolam effects were independent of cycle phase across most other measures.

4. Discussion

The results of this study clearly demonstrate that triazolam functioned as a discriminative stimulus in healthy women and that sensitivity to the discriminative stimulus effects of triazolam was influenced by menstrual cycle phase. The discriminative stimulus effects of the 0.12 mg/70 kg test dose were enhanced during the mid-luteal phase, compared to the early follicular phase, of the menstrual cycle. These results, along with a growing body of literature, support the hypothesis that progesterone modulates the behavioral effects of GABAergic drugs (e.g., Grant et al., 1997; Green et al., 1999; Kroboth et al., 1985; McAuley et al., 1995).

Menstrual cycle phase effects were detected with drug discrimination and with the stimulant subscale of the ARS. Previous research using drug discrimination with nonhuman primates showed enhanced sensitivity to alcohol effects during the mid-luteal phase (Grant et al.,

1997, Green et al., 1999); however, self-report measures of sedative drug effects have not typically changed across menstrual cycle phases in clinical studies (e.g., Holdstock & de Wit, 2000; Rukstalis & de Wit, 1999). These data suggest that drug discrimination may be more sensitive to menstrual cycle phase modulation of sedative drug effects than self-report measures alone. It has been suggested that drug discrimination may be more sensitive to drug action at the receptor level, such that drugs with similar pharmacological profiles share similar discriminative effects, while subjective effect measures may be more likely to reflect interoceptive cues engendered by non-pharmacological states (Kelly et al., 2003). The discordance between the discriminative stimulus and subjective effects of drugs has been reported across several drug classes (Preston & Bigelow, 1991; Chait et al., 1986; Kelly et al., 2003) and may be in part influenced by verbal conditioning history, contingencies associated with responding, or training conditions.

During control sessions, which were scheduled without regard to menstrual cycle phase, the 0 mg/70 kg dose engendered consistent responding on the non-drug option. Surprisingly, during the early follicular phase of test sessions, placebo engendered triazolam-appropriate responding in three of seven participants. The potential influence of several factors was examined, including the number of days post menses of testing, prior drug use history, rate of acquisition of the triazolam discrimination, and menstrual cycle phase during control sessions, but none served as a reliable predictor of placebo responding during the early follicular phase.

There were a few notable limitations to the current study. First, although cycle phases were determined using the onset of menses and objective measures of ovulation among women with regular menstrual cycles, plasma hormone levels were not measured. The correlations between serum levels of estradiol and progesterone and drug response have been demonstrated (White et al., 2002; Justice & de Wit, 2000; de Wit & Rukstalis, 1997). Second, the Sampling and Control Phases of the discrimination training were conducted without regard to menstrual cycle phase. It is possible that varied hormone levels during sampling and control phases may have influenced either rate of acquisition of the discrimination or results obtained during the test phase. However, we were unable to identify any factors, related to cycle phase or otherwise, that were associated with individual differences in the rate of acquisition of the discrimination. Furthermore, the proportion of participants acquiring the drug discrimination (7 of 12) is comparable to that in other triazolam drug discrimination studies (Vansickle & Rush, 2006; Rush et al., 2003; Rush et al., 2000; Kamien et al., 1997). Third, although a within-subject design was employed, the sample size was modest, and the sensitivity of self-report and other measures to cycle phase effects might have been established with a larger sample. Despite these limitations, the results of the present study demonstrated the influence of menstrual cycle phase on the discriminative stimulus effects of triazolam in healthy women.

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Figure 1.

Average triazolam-appropriate responding as a function of triazolam dose and menstrual cycle phase from assessments occurring between 90 and 150 minutes post dose. The left panel displays responding during the Control Phase, during which training doses were administered. Data points represent means ($\pm$ SEM denoted by the error bars) of seven participants. The asterisk indicates a significant difference ($p \leq .05$) across cycle phase.

Table 1

Significant key *F*-values of measures for which a significant main effect or interaction was obtained.

MEASURE	DOSE DF (3, 90)	CYCLE DF (1, 90)	C X D DF (3, 90)	DOSE X TIME DF (15, 90)	C X T DF (5, 90)
ARS: STIMULANT					
SEDATIVE	6.67**		4.16*	4.37***	
VAS: HIGH	3.19*			2.03*	
SEDATED	4.51*			2.87**	
DRUG EFFECT	4.35*			3.94***	
LIKE EFFECT				1.84*	
HUNGRY					2.75*
DSST: TRIAL RATE	4.62*			2.25**	
% CORRECT					
VITALS: HEART RATE	3.49*	36.54***		2.16**	
SYSTOLIC BP					
DIASTOLIC BP	4.45*	7.09*			4.00**

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$