Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 379–383, 1999

0091-3057/99/$–see front matter

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PII S0091-3057(99)00057-X

The Influence of Menstrual Cycle Phase on Sensitivity to Ethanol-Like Discriminative Stimulus Effects of $GABA_A$-Positive Modulators

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GREEN, K. L., A. V. AZAROV, K. T. SZELIGA, R. H. PURDY AND K. A. GRANT. *The influence of menstrual cycle phase on sensitivity to ethanol-like discriminative stimulus effects of $GABA_A$-positive modulators.* PHARMACOL BIOCHEM BEHAV **64**(2) 379–383, 1999.—Previous studies showed that sensitivity to the ethanol-like discriminative stimulus effects of allopregnanolone and ethanol are enhanced during the luteal phase of the menstrual cycle when progesterone levels peak in monkeys trained to discriminate 1.0 g/kg ethanol. The present study further explored the influence of the menstrual cycle phase on the discriminative stimulus effects of ethanol, allopregnanolone, and midazolam. Female adult cynomolgus monkeys (*Macaca fascicularis*) were trained to discriminate 1.0 g/kg ethanol ($n = 3$) or 2.0 g/kg ethanol ($n = 4$) (20% w/v; IG) from water (IG). A cumulative dosing procedure was used to test discriminative stimulus effects of ethanol (0.5–2.5 g/kg; IG) and the ethanol-like discriminative stimulus effects of allopregnanolone (0.1–1.0 mg/kg; IV) or midazolam (1.0–17 mg/kg; IG) during the follicular vs. luteal phase of the menstrual cycle. In the 2.0-g/kg group, sensitivity to the ethanol- like effects of allopregnanolone was increased during the luteal vs. follicular phase in two of three monkeys. In contrast, average sensitivity to ethanol was not different in the luteal compared to the follicular phase in the 2.0-g/kg group. Finally, there was no difference in sensitivity to midazolam between the follicular and luteal phases in monkeys trained with either 2.0 g/kg or 1.0 g/kg ethanol. Overall, the ethanol-like discriminative stimulus effects of midazolam are not sensitive to the menstrual cycle phase. In addition, there was less influence of the menstrual cycle phase on allopregnanolone and ethanol sensitivity in a 2.0-g/kg compared to a 1.0-g/kg ethanol training dose. © 1999 Elsevier Science Inc.

Ethanol Cynomolgus monkeys Midazolam Drug discrimination Menstrual cycle Progesterone 3α-Hydroxy-5α-pregnan-20-one Allopregnanolone

ONE of several receptor systems believed to be involved in mediating the discriminative stimulus effects of ethanol is the $GABA_A$ receptor complex (7). Although ethanol is believed to function as a positive modulator of $GABA_A$, its direct mechanism of action is uncertain. One class of ligands that modulates activity at $GABA_A$ receptors is the neurosteroids. Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is a neurosteroid that acts as a positive modulator of $GABA_A$ receptors (14). Drug discrimination studies have shown that allopregnanolone substitutes for the discriminative stimulus effects of ethanol in rats (2) and monkeys (8,9). One compelling aspect of this finding is that the production of allopregnanolone is known to fluctuate with endogenous levels of its steroid precursor progesterone (3,14). In women, ovulation stimulates ovarian production of progesterone during the luteal phase of the menstrual cycle, and then the progesterone level drops off during the premenstrum, remaining low over the follicular phase of the next menstrual cycle (18). This pattern of fluctuation in hormone levels over the course of the menstrual cycle is also seen in adult macaque monkeys (5,6).

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In a recent study, the menstrual cycle phase differentially affected sensitivity of ethanol and allopregnanolone to produce ethanol-like discriminative stimulus effects (9). Specifically, lower doses of ethanol and allopregnanolone were required to produce complete substitution for 1.0 g/kg ethanol in the luteal phase compared to the follicular phase of the menstrual cycle in female cynomolgus monkeys (*Macaca fascicularis)* (9). Thus, endogenous levels of allopregnanolone related to the menstrual cycle phase appear additive to the behavioral effects of exogenously administered allopregnanolone and ethanol.

The purpose of the present study was twofold. The first objective was to extend the findings of menstrual cycle influence on the discriminative stimulus effects of $GABA_A$ modulators to the benzodiazepine receptor. Midazolam was selected because it positively modulates $GABA_A$ channel function, although at a receptor site that is distinct from the neurosteroid site, and not present on all $GABA_A$ receptor systems. The second objective stems from previous drug discrimination data showing that training dose influences the potency and efficacy of $GABA_A$-positive modulators to produce ethanol-like discriminative stimulus effects (10,11). The present study reexamined the effects of the menstrual cycle phase on sensitivity to the discriminative stimulus effects of ethanol and allopregnanolone with a higher training dose of ethanol.

METHOD

Adult female cynomolgus monkeys were trained to discriminate either 1.0 g/kg ($n = 3$) or 2.0 g/kg ($n = 4$) ethanol from water in a two-lever operant procedure with food reinforcement. The details of this training are described in an earlier publication (8), but it should be noted that the monkeys were trained 5 days per week, regardless of menstrual cycle phase. Briefly, ethanol (1.0 or 2.0 g/kg) or a comparable volume of water was administered by intragastric intubation (IG) 30 min prior to the start of a training session. Consecutive responding on the infusion appropriate lever under a fixed-ratio (FR) schedule of reinforcement resulted in the delivery of a 1-g banana-flavored pellet, and incorrect responses reset the FR requirement for the correct lever. For all animals in the 1.0-g/kg group and monkeys 2835 and 3220 in the 2.0-g/kg group, FR was set at 30. Monkeys 2429 and 3220 in the 2.0-g/kg training group responded under an FR 50 schedule. Training sessions terminated following the delivery of 10 pellets or 30 min. Criteria for acquiring the discrimination were five consecutive sessions in which 1) at least 70% of the responses in the first ratio occurred on the condition-appropriate lever, and 2) at least 90% of the total number of responses in the session occurred on the condition-appropriate lever.

Following training, a cumulative dosing procedure was used to assess changes in the potency of the discriminative stimulus effects of ethanol (0.5–2.5 g/kg; IG), or the ethanol-like discriminative stimulus effects of allopregnanolone (0.1–1.0 mg/kg; IV) or midazolam (1–17 mg/kg; IG) during the follicular or luteal phase of the menstrual cycle. All drugs were tested in the 2.0-g/kg training group, and midazolam was tested in the 1.0-g/kg training group [ethanol and allopregnanolone determinations have been reported previously (9)]. The cumulative dosing procedure began with a drug vehicle trial followed by four additional doses of drug (15–20 ml/injection) administered 20 min apart. During these test trials, consecutive responses on either lever that completed the FR requirement were reinforced. Each trial ended following the delivery of three reinforcers or 5 min, whichever occurred first. Complete substitution for the discriminative stimulus effects of ethanol was operationally defined as occurrence of 80% or more of the total number of responses occurring on the ethanol-appropriate lever. A control cumulative-dosing test using repeated water gavages was performed to address discrimination of motor effects due to the experimental design. In all subjects, cumulative water administration resulted in nearly exclusive responding on the water-appropriate lever (>90%) and did not affect rates of responding.

The menstrual cycle was monitored over a period of 36 months prior to the start of this experiment. Based on individual cycle patterns, the menstrual day was estimated by counting backward from the first day of menses (referred to as reverse cycle day). A 3-ml venous blood sample was drawn from subjects within 24 h of test drug cumulative dose–response determinations, and verification of the follicular and luteal phase was performed by assaying for serum progesterone levels (ng/ml) as done previously (9).

Discrimination data are reported as the percentage of total responding that occurred on the ethanol-appropriate lever (responses on the ethanol-appropriate lever/total number of responses on both levers). ED_{50} values for drug substitution were determined by linear regression of the ascending limb of the stimulus generalization curve, using at least three points, or by simple extrapolation in the few cases where only two points were available [see (12)]. These data are presented as mean ± SEM.

Ethanol (100%) was diluted with tap water to a concentration of 20% (w/vol). Allopregnanolone was synthesized by the procedure of Purdy et al. (15) and suspended in sterile intralipid fat emulsion (20%, Kabi Pharmacia, Clayton, NC). The midazolam base, which was diluted in saline, was provided by Hoffmann–LaRoche Ltd.

RESULTS

Over the 36 months the menstrual cycles were monitored; the average length of the cycle did not differ between monkeys trained to discriminate 1.0 g/kg (range 27–31 days) and 2.0 g/kg (range 28–34 days) ethanol. For purposes of the present study design, the follicular phase encompassed reverse cycle days 18–33 and the luteal phase encompassed reverse cycle days 7–13. Serum progesterone concentrations (ng/ml) were indicative of the menstrual cycle phase, with 10- to 50-fold higher levels in the luteal compared to the follicular phase. In monkeys trained to discriminate 1.0 g/kg ethanol, average follicular phase progesterone levels were 0.22 ± 0.01 ng/ml, and average luteal phase progesterone levels were 11.3 ± 0.76 ng/ml. In monkeys trained to discriminate 2.0 g/kg ethanol, average follicular phase progesterone levels were 0.48 ± 0.14 ng/ml and average luteal phase progesterone levels were 7.7 ± 1.55 ng/ml.

Midazolam resulted in complete substitution for ethanol in all monkeys tested (see Fig. 1). In monkeys trained with 1.0 g/kg ethanol, the average ED_{50} for midazolam substitution was not different in the follicular phase (2.0 ± 0.6 mg/kg) compared to the luteal phase (2.1 ± 0.7 mg/kg) of the menstrual cycle. Similarly, in monkeys trained with 2.0 g/kg ethanol, the average ED_{50} for midazolam substitution was not different in the follicular phase (3.5 ± 0.6 mg/kg) compared to the luteal phase (3.8 ± 0.9 mg/kg) of the menstrual cycle. However, there was a difference in sensitivity to the ethanol-like effects of midazolam as a function of training dose. Average ED_{50} of midazolam combined across the menstrual cycle phase was lower in 1.0-g/kg ethanol monkeys (2.1 ± 0.4 mg/kg) com-

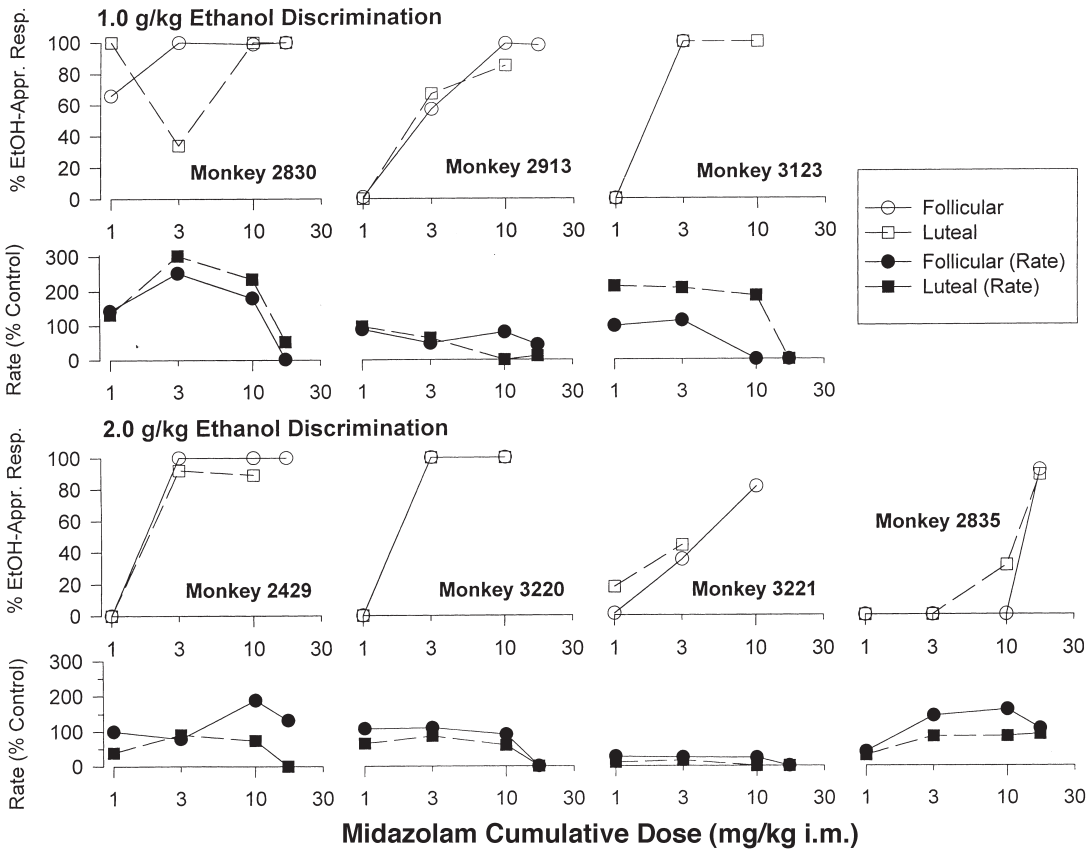

FIG. 1. Percentage of total session responding (open symbols) on the ethanol-appropriate lever and correlated response rate (% of saline control; filled symbols) following the cumulative administration of midazolam in animals trained to discriminate 1.0 g/kg (upper panels) and 2.0 g/kg (lower panels) ethanol during the follicular (circle) or luteal (square) phase of the menstrual cycle. See text for details regarding the cumulative dosing procedure.

pared to 2.0-g/kg ethanol monkeys (3.6 ± 0.5 mg/kg). Rates of responding were highly variable between monkeys, but were apparently unrelated to the menstrual cycle phase.

Cumulative doses of ethanol resulted in complete substitution for 2.0 g/kg ethanol in all monkeys tested. Sensitivity to ethanol's discriminative stimulus effects varied among individual monkeys and over the course of the menstrual cycle (Fig. 2). The mean ED_{50} for producing ethanol substitution was similar between the follicular phase (1.6 ± 0.2 g/kg ethanol) and the luteal phase (1.2 ± 0.1 g/kg ethanol) of the menstrual cycle. Monkeys 2429 and 2835 were more sensitive, while monkey 3221 was less sensitive to ethanol's discriminative stimulus effects during the luteal phase of the menstrual cycle. There was no apparent influence of menstrual cycle phase on sensitivity to response rates following the cumulative doses of ethanol.

Cumulative doses of allopregnanolone resulted in complete substitution for 2.0 g/kg ethanol in two of three monkeys tested (Fig. 2). For both of these monkeys, allopregnanolone was more potent in producing complete ethanol substitution in the luteal compared to the follicular phase of the menstrual cycle. Notably, allopregnanolone did not produce substitution for ethanol in monkey 3220 during the follicular phase, a finding that was also present in one of the monkeys trained to discriminate 1.0 g/kg ethanol (9). Overall, these data indicate that the potency of allopregnanolone to substitute for ethanol was least efficacious and potent during the follicular phase. Rates of responding following allopregnanolone administration were unrelated to menstrual cycle phase.

DISCUSSION

The results show that substitution of allopregnanolone for the discriminative stimulus effects of ethanol varies with menstrual cycle phase in two of three monkeys trained to discriminate 2.0 g/kg ethanol. Allopregnanolone was more potent and efficacious when tested during the luteal phase of the menstrual cycle. These results replicate previous findings with a 1.0-g/kg ethanol training dose (9). When the present data are combined with prior data (9), five of six monkeys tested to date are more sensitive to ethanol-like discriminative stimulus effects in the luteal compared to the follicular phase of the menstrual cycle, independent of training dose. These results suggest that endogenous allopregnanolone levels associated with the menstrual cycle phase are additive to exogenously administered allopregnanolone in producing subjective effects similar to those of ethanol.

In contrast to previous results, mean sensitivity to ethanol did not differ as a function of menstrual cycle. However, two of four monkeys in the present study showed increased sensitivity to ethanol in the luteal phase of their menstrual cycle. However, the attenuated influence of the menstrual cycle on ethanol sensitivity in the higher training dose could be due to

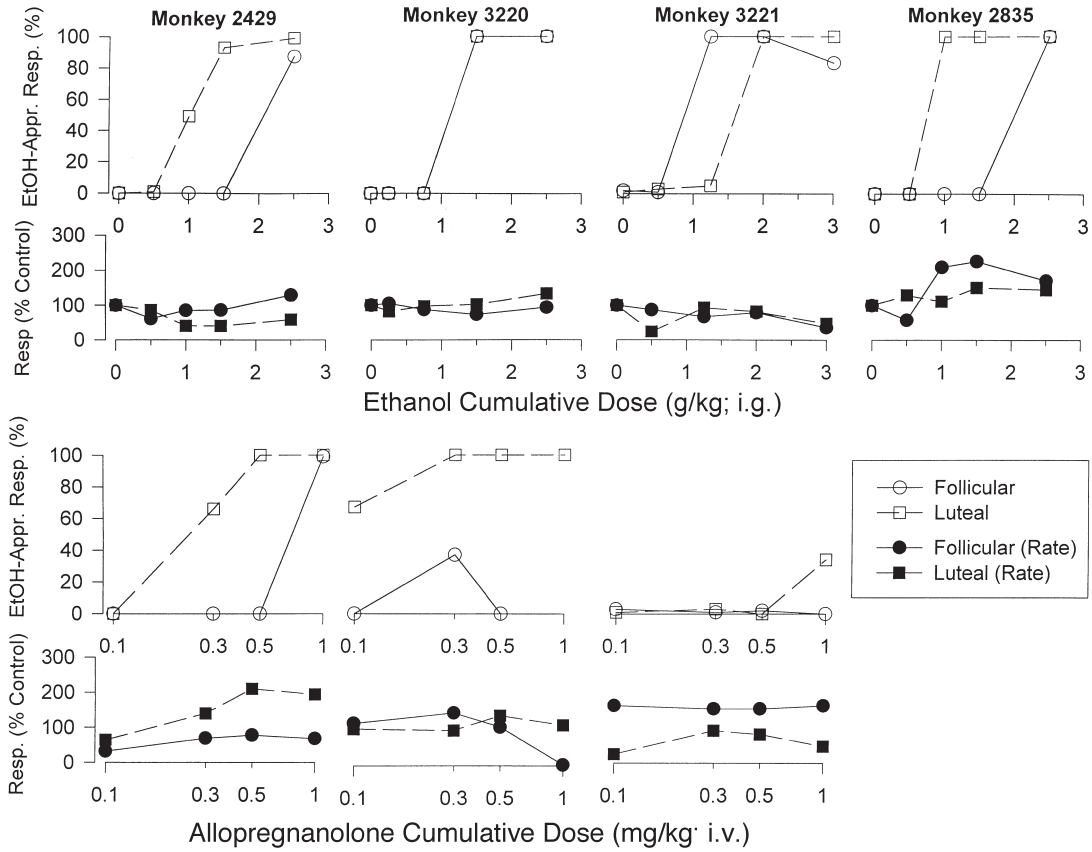

FIG. 2. Percentage of total session responding (open symbols) on the ethanol-appropriate lever and correlated response rate (% of saline control; filled symbols) following the cumulative administration of ethanol (upper panels) or allopregnanolone (lower panels) in animals trained to discriminate 2.0 g/kg ethanol during the follicular (circle) or luteal (square) phase of the menstrual cycle. See text for details regarding the cumulative dosing procedure.

a direct effect of high ethanol doses on the HPG axis function (13). For example, in this experiment, average luteal phase progesterone levels were higher in the 1.0-g/kg compared to the 2.0-g/kg trained monkeys. Lower luteal phase progesterone levels are indicative of impaired menstrual cycles (17). Nevertheless, combining present and prior data, six of eight monkeys tested to date are more sensitive to the discriminative stimulus effects of ethanol in the luteal compared to the follicular phase, independent of training dose.

Unlike ethanol and allopregnanolone (9), the menstrual cycle phase clearly did not alter sensitivity to the benzodiazepine midazolam in animals trained with either a 1.0-g/kg or 2.0-g/kg ethanol dose. These data are similar to findings in humans who showed no difference in the subjective effects of the benzodiazepine triazolam across the menstrual cycle (4). In animal models, the discriminative stimulus effects of midazolam appear to be mediated via benzodiazepine receptors located on a subset of $GABA_A$ receptor channels (16). Likewise, the ethanol-like discriminative stimulus effects of midazolam are blocked by the benzodiazepine receptor antagonist flumazenil (1). Thus, these data support a separate receptor mechanism in mediating ethanol-like subjective effects that are sensitive to cyclic fluctuation in levels of progesterone and allopregnanolone. Because studies indicate a neurosteroid site on $GABA_A$ receptors that is distinct from the benzodiazepine site, the present data suggest the discriminative stimulus effects of ethanol that are enhanced during the luteal phase may be mediated by neurosteroid binding sites. Additional studies are needed to address whether other physiological states associated with $GABA_A$ activity, such as stress, increase sensitivity to the effects of ethanol through the neurosteroid receptor. Finally, the difference in potency of midazolam to produce ethanol-like discriminative stimulus effects as a function of ethanol training dose is similar to previous findings with pentobarbital (10, 11). These data are consistent with the concept that ethanol's discriminative stimulus effects mediated by $GABA_A$ neurotransmission are more prominent at lower training doses.

ACKNOWLEDGEMENTS

Care and treatment of the subjects used in this study were in accord with state and federal guidelines. The experimental protocol was approved by the Wake Forest University Animal Care and Use Committee. This research was supported in part by grants from the National Institute on Alcohol Abuse and Alcoholism RO1 AA10009, T32 AA07565, and P5O AA06420. Midazolam was provided by Dr. P. Weber of Hoffmann–LaRoche Ltd. (Basel, France). Serum progesterone levels were determined at the Assay Services of Yerkes Regional Primate Research Center of Emory University under the direction of Dr. M. Wilson.

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