Neuro
-endocrinology

Neuroendocrinology 2000;71:51–59

Received: July 7, 1999
Accepted after revision: October 28, 1999

Acute Effects of Estradiol Pretreatment on the Response to *d*-Amphetamine in Women

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Key Words
Gonadal steroids · Menstrual cycle · Amphetamines · Clinical neuroendocrinology · Behaviour

Abstract
Little is known about the interactions between ovarian hormones and responses to psychoactive drugs in humans. Preclinical studies suggest that ovarian hormones such as estrogen and progesterone have direct and indirect central nervous system actions and that these hormones can influence behavioral responses to psychoactive drugs. In the present study, we assessed the subjective and physiological effects of *d*-amphetamine (AMPH; 10 mg p.o.) after pretreatment with estradiol. Two groups of healthy, regularly cycling women participated in two sessions scheduled during the early follicular phases of two menstrual cycles. One group received estradiol patches (Estraderm TTS; 0.8 mg) which elevated plasma estradiol levels to approximately 750 pg/ml on both sessions; the other group received placebo patches on both sessions. Both groups received AMPH (10.0 mg) and placebo in a randomized and counterbalanced order on the two sessions. Dependent measures included self-report questionnaires, physiological measures, and plasma hormone levels. Most of the subjective and physiological effects of AMPH were not affected by acute estradiol treatment. Nevertheless, estradiol pretreatment increased the magnitude of the effects of AMPH on subjective ratings of 'pleasant stimulation' and decreased ratings of 'want more'. Also, estradiol produced some subjective effects when administered alone: It increased subjective ratings of 'feel drug', 'energy and intellectual efficiency', and 'pleasant stimulation'. These results provide limited evidence that the stimulating effects of AMPH are increased by acute estradiol pretreatment.

Introduction

We recently reported that the subjective and behavioral effects of *d*-amphetamine (AMPH) varied across the menstrual cycle, possibly related to relative levels of estrogen and progesterone [1]. Subjects reported feeling more 'high', 'energetic and intellectually efficient', and 'euphoric' when AMPH was administered during the follicular phase as compared with the luteal phase. Subjects also reported liking and wanting AMPH more during the follicular phase than during the luteal phase. The differential

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effects at different cycle phases suggested that the subjective effects of AMPH may be related to the levels of estrogen and progesterone. The follicular phase is characterized by initially low and then gradually rising levels of estrogen with very low levels of progesterone, whereas the luteal phase is characterized by moderate to high levels of both hormones. Further analyses of our data showed that subjects' responses during the follicular phase were related to plasma levels of estrogen. AMPH-induced increases in 'euphoria' and 'energy and intellectual efficiency' were positively correlated with estrogen levels. We speculated that potential interactions between estrogen and AMPH during the luteal phase may have been masked by the presence of progesterone [e.g., 2]. These findings suggest that in the absence of progesterone, estrogen may enhance responses to a stimulant drug in normal women.

The finding of an interaction between estrogen and AMPH is consistent with studies in laboratory animals. Rats exhibit the greatest behavioral response to AMPH when estrogen levels are high relative to progesterone levels, during late proestrus or early estrus [e.g., 3]. For example, female rats exhibit more stereotypy [3] and rotational behavior [4] during estrus than during diestrus. Rats also release more dopamine (DA) in response to AMPH when the estrogen levels are high relative to the progesterone levels [e.g., 3, 5]. The enhancement of responses to AMPH may be mediated by an effect of estrogen on DA. The dopaminergic activity increases in response to exogenous administration of estradiol in rats. For example, in ovariectomized rats, estradiol increases dopamine synthesis [6, 7], turnover and release [e.g., 5, 8, 9], and DA receptor density [10, 11]. Estrogen also decreases monoamine oxidase activity, thereby regulating the degradation of DA [12]. Furthermore, estrogen modulates the presynaptic DA transporter in female rats [13], dose dependently decreasing the affinity of the DA transporter, thus preventing reuptake [14]. These data suggest that estrogen may increase responses to AMPH by increasing the activity of the DA system.

There are several difficulties in studying drug-hormone interactions using spontaneous hormone variations across the menstrual cycle. First, the approach is fundamentally correlational. A variety of hormonal and physiological changes occur concurrently across the menstrual cycle, making it impossible to identify individual causal variables [15, 16]. Second, there is considerable inter- and intrasubject variability in the timing and magnitude of hormonal events, making it difficult to conduct controlled studies. One way to circumvent these problems is by administering specific hormones exogenously. This provides the investigator with greater control of the levels and timing of the hormonal environment. In women with normal menstrual cycles, the simplest and most convenient time to administer exogenous hormones is the early follicular phase. This phase is readily identified by the onset of menses, it lasts several days allowing some flexibility in scheduling of sessions, and it is associated with very low levels of endogenous ovarian hormones.

The present study examined the interaction between estrogen and AMPH in women by administering 17β-estradiol to subjects during the early follicular phase of their cycle. It was hypothesized that estradiol treatment would enhance the subjective and behavioral effects of AMPH. To our knowledge, this is the first study to assess the subjective and physiological effects of an acute dose of estradiol and its interaction with AMPH in normal, cycling women.

Methods

Design

Healthy young women were randomly assigned to one of two groups: the placebo patch group (PP; n = 12) or the estradiol patch group (EP; n = 12). Each subject participated in two sessions scheduled once a month during the early follicular phases of two (or three, if they missed a session) consecutive menstrual cycles. The EP group received estradiol transdermal patches (0.8 mg Estraderm TTS) on both sessions; the PP group received placebo patches on both sessions. All subjects received AMPH (10 mg p.o.) on one session and placebo on the other session, with the order counterbalanced across subjects. The 0.8-mg dose of estradiol was selected because it is known to raise plasma levels to supraphysiological levels [Justice and de Wit, unpubl. data] which would maximize our chance of seeing an effect of estradiol. The dose of AMPH was selected because it is known to produce reliable, but modest, subjective effects, thereby allowing us to detect estradiol-dependent increases or decreases in effect [17]. Subjective, behavioral, and physiological responses were assessed at regular intervals during each session.

Subject Recruitment and Screening

Twenty-three women aged 18–35 years were recruited from the university and surrounding community via posters, advertisements in newspapers, and by word of mouth referrals. Initial eligibility was ascertained in a telephone interview. Eligible candidates reported to the laboratory to complete standardized self-report questionnaires including the Symptom Checklist 90 [18] and a health questionnaire containing items of general health and drug and alcohol use. Screening included a physical examination, an ECG, a semistructured psychiatric interview, and urine pregnancy tests. Exclusion criteria were: irregular menstrual cycles, severe premenstrual syndrome or any menstrual cycle dysfunction, use of hormonal contraceptives, lactation, pregnancy or plans for pregnancy, and endocrine, medical, or axis I [19] psychiatric disorders including substance use disorders. The procedure was approved by the Institutional Review Board at the University of Chicago Hospital.

Before the study, subjects read and signed the consent form, and any questions about it were answered. The consent form outlined the procedures to be followed and listed the classes and possible effects of any drugs that subjects might receive. For blinding purposes, the subjects were told that on any session they might receive a stimulant, tranquilizer, hormone, or placebo. They were instructed to abstain from alcohol and other drugs before the sessions, and this was verified using breath alcohol levels (BAL) and urine toxicology tests. BALs were determined prior to each session using an Intoximeter Breathalyzer. No BAL reading was positive. Urine samples were obtained prior to each session and screened for pregnancy. In addition, one of the urine samples was randomly selected for a urine toxicology screen to verify the nonuse of stimulants, barbiturates, opioids, and benzodiazepines. No urine toxicology screen was positive. The subjects agreed not to take any other drugs for 12 h before and 6 h following each session.

Laboratory Environment

Sessions were conducted in comfortably furnished rooms in the human Behavioral Pharmacolgy Laboratory of the Department of Psychiatry. The subjects were tested individually and were allowed to bring in their own recreational materials.

Procedure

During an orientation session before the first session, the subjects were given envelopes containing either placebo or estradiol patches for both sessions and instructions on how to apply them at home. Estradiol and placebo patches were similar in appearance, and the patch presentation was double-blind. The subjects also received questionnaire packets to complete prior to applying the patches on each session. The subjects participated in two 4.5-hour sessions conducted once a month from 08.30 to 13.00 h. Each session was scheduled during the early follicular phase, 2–7 days after the 1st day of menstruation. The subjects were instructed to fast overnight prior to each session. At 06:00 h before each session, before coming to the laboratory, the subjects applied the patches (estrogen or placebo) to their buttocks or abdomen. The patches were applied at 06.00 h to allow 5 h for absorption. The estradiol pretreatment was scheduled so that peak plasma levels would coincide with peak AMPH levels: both were expected to peak at around 11.00 h. Prior to applying the patches, the subjects completed baseline self-report mood questionnaires. Immediately after applying the patches, the subjects telephoned the research coordinator to record the exact time of patch application. At 08.30 h, the subjects reported to the Clinical Research Center to provide a blood sample for precapsule estradiol and progesterone assays. Blood samples were centrifuged, and the serum was frozen at –70 °C until the hormone assays were conducted. The subjects then proceeded to the Human Behavioral Pharmacology Laboratory for the 4.5-hour session. Upon arrival, they provided urine samples for pregnancy tests and completed baseline measures of their mood (see below). In addition, their heart rate, blood pressure, and temperature were recorded. At 09.00 h, after a negative pregnancy test was confirmed, the subjects ingested capsules containing AMPH (10 mg) or placebo with 100 ml of water. AMPH was placed in opaque, colored gelatin capsules (size 00) and were packed with dextrose filler. The placebo capsules contained only dextrose. The subjects received AMPH on one session and placebo on the other. Half of the subjects received AMPH first, and the other half received placebo first. Drug presentations were double-blind. After taking the capsule, the subjects were free to relax in the laboratory. They completed mood questionnaires, and vital signs were recorded 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h after ingesting the capsule. At 12.00 h the subjects were escorted to the Clinical Research Center where another blood sample was collected for postcapsule estradiol and progesterone assays. The subjects left the laboratory shortly after 13.00 h.

Dependent Measures

Subjective effects of estradiol and AMPH were assessed with the Profile of Mood States (POMS) [20], the Addiction Research Center Inventory (ARCI) [21], and two visual analog questionnaires, the Stimulant Sedative Questionnaire (SSQ) [Justice and de Wit, in preparation] and the Drug Effects Questionnaire (DEQ). Visual analog scales are 10-cm lines tagged with an adjective and labeled at either end with opposites such as 'not at all' and 'extremely'. Subjects respond by placing a vertical tick mark along the continuum at a location reflective of their current mood or state. The POMS consists of 72 adjectives commonly used to describe momentary mood state. The subjects indicate how they feel at that moment in relation to each of the adjectives on a 5-point scale ranging from 'not at all' (0) to 'extremely' (4). The 49-item ARCI is a true-false questionnaire with five empirically derived scales: A (AMPH-like group, stimulant effects), BG (benzedrine group, energy and intellectual efficiency), MBG (morphine-benzedrine group, euphoric effects), LSD (lysergic acid diethylamide group, dysphoric effects, somatic complaints), and PCAG (pentobarbital-chlorpromazine-alcohol group, sedative effects). The SSQ is a 22-item questionnaire with four factor-analyzed measures: 'pleasant stimulation', 'unpleasant stimulation', 'pleasant sedation', and 'unpleasant sedation'. 'Pleasant stimulation' includes the visual analog scales 'alert', 'focused', 'outgoing', 'energetic', and 'lively'. 'Unpleasant stimulation' includes the visual analog scales 'restless', 'anxious', 'jittery', 'on edge', 'uneasy', and 'nervous'. 'Pleasant sedation' includes the visual analog scales 'calm', 'relaxed', 'peaceful', 'contented', and 'mellow'. 'Unpleasant sedation' includes the visual analog scales 'tired', 'sluggish', 'worn out', 'drowsy', 'slow', and 'heavy'. The DEQ assessed the subjective states 'feel drug', 'feel high', 'like drug', and 'want more'. The primary dependent measures were the 'pleasant stimulation' scale of the SSQ, the A (AMPH-like effects) and BG (energy and intellectual efficiency) scales of the ARCI, and the 'feel drug' scale of the DEQ. These measures have been shown to be sensitive to the effects of a variety of psychoactive drugs, including stimulants [22].

Plasma samples were assayed for estradiol and progesterone at the University of Chicago Endocrinology Laboratory. Plasma estradiol levels were measured using the IMx (Abbott Laboratories) estradiol assay which is based on the microparticle enzyme immunoassay technology. It has a sensitivity of 25 pg/ml, an interassay coefficient of variation $< 10\%$, and a very low cross-sensitivity with other compounds. The plasma progesterone levels were measured using the coat-a-count progesterone procedure (Diagnostic Products). This procedure has a sensitivity of 0.02 ng/ml, low and uniform coefficients of variation in retests, and a low cross-reactivity to other compounds.

Data Analysis

To determine whether the responses to AMPH were different in the PP group and in the EP group, three-way repeated-measures Anovas were conducted on each dependent measure with within-subjects factors of drug (AMPH or placebo) and time (prepatch and repeated measures within sessions) and a between-subjects factor of

group (PP or EP). To minimize the influence of variability between subjects prior to patch administration, scores on ARCI, POMS, and visual analog scale measures were calculated as change from baseline (i.e., prepatch score was subtracted from each subsequent time point). For the DEQ, which was not administered prior to patch administration, only raw scores were used. For all analyses, F values were considered significant at $p < 0.05$. Fisher-Hayter post hoc comparisons were conducted when significant group by drug by time interactions were observed. All analyses were conducted with SPSS for Macintosh version 6.1.1.

Results

Subject Characteristics

Twenty women completed the study and provided usable data (EP n = 11; PP n = 9). Two subjects initially assigned to the PP group did not complete the study, one due to scheduling problems, the other due to pregnancy. One subject in the EP group did not complete the study due to scheduling problems. One subject in the PP group completed the study, but did not complete the forms properly. Her data are not included in these analyses. The groups did not differ significantly on any demographic parameter (table 1).

Table 1. Demographic characteristics of each group (mean ± SD)

	PP group (n = 9)	EP group (n = 11)
Age, years	25.8 ± 5.3	22.7 ± 3.2
Height, cm	166.1 ± 6.4	165.5 ± 6.3
Weight, kg	63.1 ± 5.3	57.2 ± 4.3
Alcohol, drinks/week	2.7 ± 3.7	1.8 ± 1.4
Cigarettes/day[a]	0.2 ± 0.7	0.8 ± 2.1
Caffeine, drinks/week	7.6 ± 8.9	7.7 ± 8.7
Length of menstrual cycle, days	29.1 ± 2.4	28.2 ± 1.1

The groups did not differ on any measure.

[a] Mean cigarettes per day among subjects (n = 4) who reported any use of cigarettes.

Hormone Levels

Table 2 shows the mean, standard deviation, minimum, and maximum levels of estradiol during the drug and placebo sessions 150 and 360 min after patch administration. These values show that the estradiol levels were significantly higher in the EP group on both sessions at both time points. There were no significant within-group differences across sessions or time points. Figure 1 shows estradiol levels 360 min after patch administration for the 9 subjects in the PP group and for the 11 subjects in the EP group. Reference values for the follicular, preovulatory, and luteal phases are also shown [16]. Figure 1 shows that the estradiol levels were within the normal range for the early follicular phase in the PP group at both time points and that the estradiol levels were approximately ten times higher than normal during the early follicular phase in the EP group at both time points.

Effects of AMPH

Regardless of the group condition, AMPH increased systolic blood pressure [$F(8, 144) = 3.10$, $p < 0.003$], heart rate [$F(1, 18) = 8.36$, $p < 0.001$], and temperature [$F(8, 144) = 3.89$, $p < 0.01$] and produced its prototypic subjective effects such as increased ratings of 'feel drug' [DEQ; $F(8, 144) = 3.45$, $p < 0.001$], AMPH-like effects [ARCI A

Table 2. Estradiol levels (pg/ml) in the EP and PP groups

	PP group (n = 9)				EP group (n = 11)			
	placebo		AMPH		placebo		AMPH	
	08.00 h	12.00 h	08.00 h	12.00 h	08.00 h	12.00 h	08.00 h	12.00 h
Mean	74.67	74.56	57.33	53.89	739.73	816.18	691.36	787.91[a]
SD	52.76	57.97	21.75	28.54	372.84	317.07	323.91	318.87
Minimum	21.00	18.00	32.00	24.00	197.00	425.00	209.00	126.00
Maximum	174.00	179.00	99.00	114.00	1,548.00	1,488.00	1,432.00	1,396.00

The measure at 08.00 h was obtained before the capsule, but 2 h after patch administration; the measure at 12.00 h was obtained 6 h after patch administration and 3 h after capsule administration.

[a] The estradiol levels were significantly higher in the EP group on both sessions at both time points; there were no differences across sessions or time points within groups.

scale; F(8, 144) = 2.27, $p < 0.05$] and energy and intellectual efficiency [ARCI BG scale; F(1, 18) = 8.88, $p < 0.01$], and vigor [POMS; F(1, 18) = 13.4, $p < 0.01$] and friendliness [POMS; F(8, 144) = 17.5, $p < 0.001$]. These results are summarized in figure 2 and table 3. Figure 2 shows subjective ratings of 'feel drug' (DEQ) 'energy and intellectual efficiency' (ARCI BG) and systolic blood pressure after AMPH in the PP and EP groups.

Effects of Estrogen

There were no significant main effects of the EP. However, post hoc analyses indicated that ratings of 'pleasant stimulation' were significantly greater in the EP group after placebo than in the PP group after placebo only at the final time point of the session. These data are presented in figure 3.

Effects of AMPH plus Estrogen

Significant group by drug by time interactions were observed on ratings of 'pleasant stimulation' [SSQ; F(144, 8) = 3.01, $p < 0.005$] and 'want more' [DEQ; F(144, 8) = 2.53, $p < 0.01$]. Figure 3 shows subjective ratings of 'pleasant stimulation' and 'want more' after AMPH in the EP and PP groups as a function of time. Ratings of 'pleasant stimulation' after AMPH were higher in the EP group than in the PP group early in the session, while later in the session the PP group showed a larger AMPH effect. On

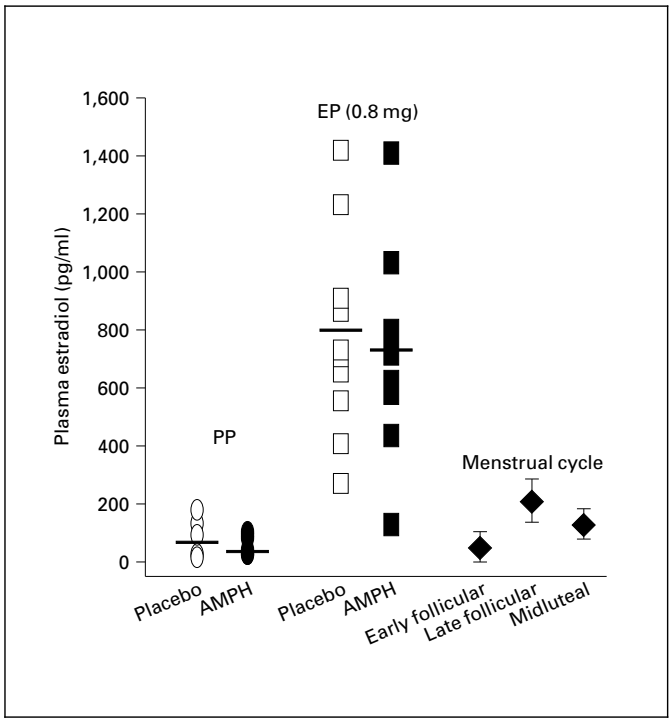

Fig. 1. Plasma levels of estradiol for the PP (circles) and EP (squares) groups 6 h after patch administration. The filled symbols represent data from AMPH sessions and the open symbols data from placebo sessions. The expected values of estradiol during the early follicular, late follicular, and midluteal phases of the menstrual are also shown (filled diamonds) [from ref. 16].

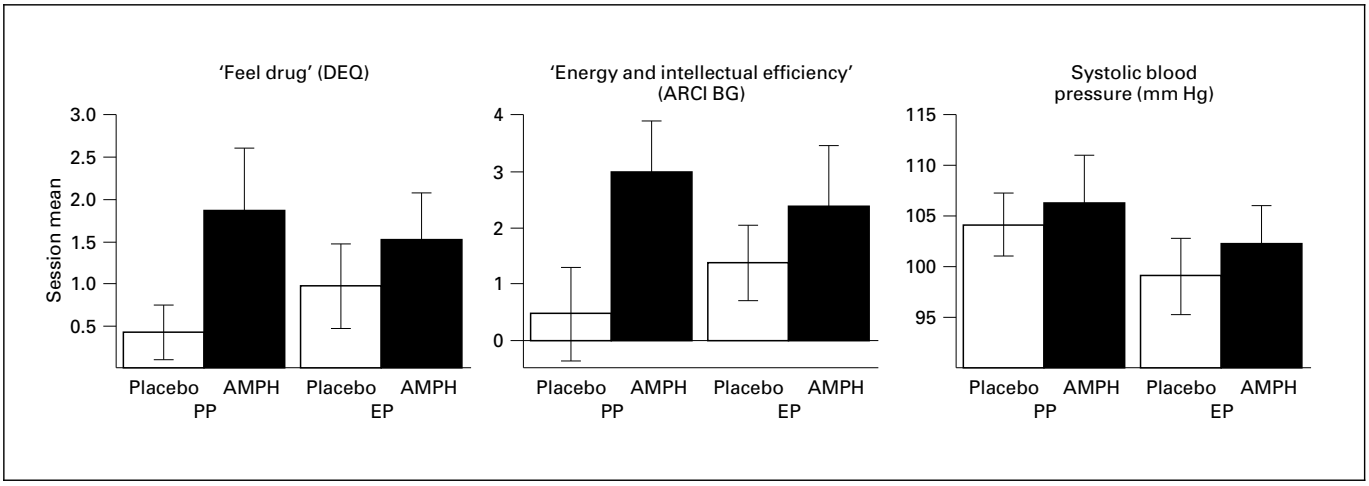

Fig. 2. Mean (SEM) for subjective ratings of 'feel drug' (DEQ; left panel), 'energy and intellectual efficiency' (ARCI BG; middle panel), and systolic blood pressure (right panel) for the PP and EP groups. Filled bars represent data from AMPH sessions. The open bars represent data from placebo sessions. The maximum score on the DEQ is 10.

Table3. Summary of significant F values for main effects of AMPH and time, interactions between AMPH and time, and interactions between estradiol, AMPH, and time

	AMPH F(1, 18)	Time F(8, 144)	AMPH·time F(8, 144)	Estradiol·AMPH·time F(8, 144)
SSQ				
'Pleasant stimulation'	4.76*	3.56***		3.01**
DEQ				
'Feel drug'	9.01**	2.53**	3.45***	
'Feel high'		2.40*	1.92 (p = 0.06)	
'Want more'			2.09*	2.53**
POMS				
Friendliness	15.7***		17.5***	
Vigor	13.4**	2.85**	1.96 (p = 0.06)	
Elation		2.57**		
Anxiety		2.03*		
Confusion		2.27*		
Fatigue			2.0*	
ARCI				
Engergy (ARCI BG)	8.88**	3.51***		
AMPH-like (ARCI A)		2.48*	2.27*	
Sedation (ARCI PCAG)	4.41*	3.51***		
Physiological				
Blood pressure				
Systolic		5.27***	3.10**	
Diastolic		2.83**		
Heart Rate	8.36**	6.99***		
Temperature		4.02***	3.89***	

There were no main effects for estradiol alone.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

the 'want more' scale, AMPH increased ratings in the PP group during the last 90 min of the session, but this effect was not evident in the EP group.

Discussion

In the present study using normal-cycling women tested during the early follicular phase, acute estradiol treatment did not change most of the subjective and physiological effects of AMPH. Nevertheless, estradiol did affect two measures of the effects of AMPH: it increased the magnitude of the effects of AMPH on subjective ratings of 'pleasant stimulation' and decreased ratings of 'want more'. Also when estradiol was administered alone, it increased subjective ratings of 'pleasant stimulation'. The transdermal administration of estradiol raised plasma levels of estradiol to about tenfold the physiologic levels normally observed during the early follicular phase. Although the effect of estradiol on the response to AMPH was unexpectedly modest, it was consistent both with preclinical data and with previous data from our laboratory using human subjects.

These data suggest that there is a partial interaction between estradiol and the subjective effects AMPH. Many of the subjective and behavioral effects of AMPH were not significantly affected by estradiol pretreatment. However, estradiol pretreatment increased feelings of stimulation after AMPH and decreased the subjects' desire for more drug. The increased ratings of stimulation are consistent with the results of our previous study comparing the effects of AMPH at the follicular and luteal phases of the cycle [1] and with those of a recent report comparing the subjective effects of smoked cocaine at different phases of the menstrual cycle in women [23]. We previously reported that the effects of AMPH were greatest during the late follicular phase, when the estrogen levels are high and progesterone levels low. Consistent with

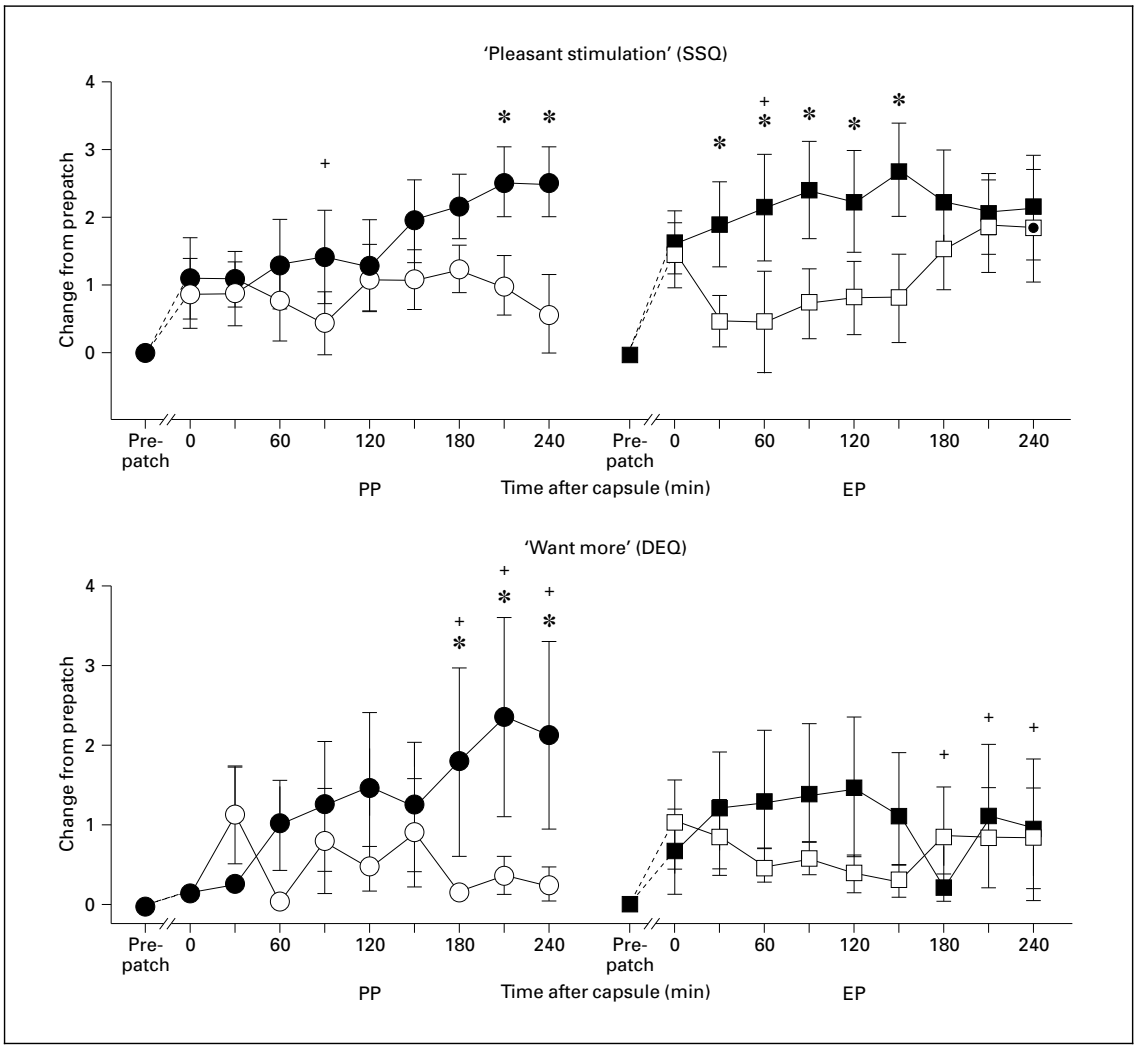

Fig. 3. Mean (SEM) subjective ratings of 'pleasant stimulation' (SSQ; top panel) and 'want more' (DEQ; bottom panel) for the PP and EP groups. The filled symbols represent data from AMPH sessions and the open symbols data from placebo sessions. Significant phase by drug by time interactions were observed on 'pleasant stimulation' (SSQ) and 'want more' (DEQ). The asterisks indicate which points are different from placebo, crosses indicate which points on AMPH sessions are different between groups, and partially filled symbols indicate which points on placebo sessions are different between groups (Fisher-Hayter post hoc test, $p < 0.05$). The maximum score on SSQ and DEQ is 10.

this, Sofuoglu et al. [23] found that another stimulant drug, cocaine, produced greater increases in stimulation in women tested during the follicular phase as compared with the luteal phase. Unfortunately, plasma estrogen and progesterone levels were not reported in the study by Sofuoglu et al. [23] to document the hormonal status. The findings from both studies [1, 23] suggest that the effects of stimulant drugs are greater when the estrogen levels are high relative to progesterone.

It has been suggested that the mechanism for interactions between stimulant drugs and ovarian hormones is through shared actions on the dopaminergic system [e.g., 3]. AMPH, cocaine, and estrogen all consistently increase DA activity [e.g., 24]. Progesterone, on the other hand, may decrease the DA activity, although the nature of this effect appears to be critically dependent on the timing of the progesterone administration [25, 26]. For example, after progesterone administration, the DA release appears

initially to increase but then later to decrease [25]. In laboratory animals, estrogen increases AMPH-induced locomotor activity, rotational behavior, and stereotypy, suggesting that this drug-hormone combination produces additive effects [e.g., 3, 4]. Again, the effects of progesterone are complicated by dose and timing characteristics, but may be opposite to those of estrogen. For example, in ovariectomized rats, continuous 40-min infusion of 2 ng/ml progesterone inhibits AMPH-stimulated DA release up to 12 h after progesterone administration. However, pulsatile infusions of the same dose of progesterone (2 ng/ml) may increase AMPH-stimulated DA release, and pulsatile infusions of either a higher (50 ng/ml) or a lower (0.2 ng/ml) dose of progesterone have no effect [25]. It has been suggested that progesterone has biphasic effects on DA release, initially increasing, but ultimately decreasing basal and AMPH-stimulated DA release [25]. When progesterone is administered to estrogen-primed rats, some investigators [27] have reported that it has no effect, whereas others [2, 28] have reported that it decreases AMPH-induced stereotypy. Estrogen and progesterone also exert different effects on the DA transporter. For example, ovariectomy, which decreases both estrogen and progesterone levels, results in an upregulation of the striatal DA transporter. Estradiol reverses this effect, but progesterone does not [13]. Likewise, estradiol, but not progesterone, decreases the affinity of the DA transporter, thereby increasing the amount of DA in the synapse [14]. In summary, estrogen and progesterone appear to exert divergent actions on DA release and DA transporter.

It is not clear why estradiol increased ratings of 'pleasant stimulation' after AMPH while decreasing the desire for more drug. The increase in 'pleasant stimulation' is consistent with a leftward shift in the AMPH dose-response curve, based on previous findings that within this dose range the magnitude of ratings of stimulantlike effects increases linearly with the dose [17]. It is possible that with ratings of desire for more drug, further increases in dose beyond a certain point lead to a decline in ratings of desire for more drug and that the estradiol treatment in our study shifted subjects' responses to the descending limb of this dose function. We do not have sufficient data to evaluate this possibility.

The effects of estradiol in the present study were limited to only one measure of stimulantlike subjective effects, suggesting that the enhancement was modest at best. Among the effects of AMPH that were not increased by estradiol were ratings of 'like drug' and 'feel high' and ratings of euphoria. In our previous study we found menstrual cycle related effects of AMPH on energy and intellectual efficiency, euphoria, and ratings of 'feel drug', 'like drug', and 'want more'. Why only increased ratings of pleasant stimulation were observed in the present study is unclear and difficult to reconcile with our previous findings [1]. One possible explanation is that estrogen enhances the response to AMPH only during certain phases of the menstrual cycle. In the present study, women were tested during the 1st week of the follicular phase, whereas in the previous study, the most marked effects were observed during the late follicular phase. It is conceivable that the central nervous system is more sensitive to the effects of estrogen during the late follicular phase than during the early follicular phase. Such phase-related differences in sensitivity could suggest alternate mechanisms for the interaction. For example, estrogen may enhance the effects of AMPH through genomic changes in the cell rather than through nongenomic actions on the DA receptor. These genomic effects occur much more slowly, possibly even days later [16]. Second, the effects of estrogen may depend on changes in DA receptor density. It is known that chronic exposure to estrogen results in increases in dopamine receptor density [10, 29, 30]. Thus, at times when estrogen levels are very low (i.e., early follicular phase), the density of DA receptors may be too low for the system to respond to estrogen in a significant way. Therefore, it is possible that exogenous estradiol administered later during the follicular phase would have resulted in greater increases in the subjective effects of AMPH.

There were several limitations to this study. First, only single doses of AMPH and estrogen were tested. A relatively low dose of AMPH was chosen because we expected to observe an increase in subjective responses after pretreatment with estrogen [17], and a relatively high dose of estradiol was chosen in order to maximize our chances of detecting estradiol-dependent increases in the subjective effects of AMPH. In future studies, it would be of interest to test a range of AMPH and estradiol doses to determine whether estradiol produces a leftward shift in the AMPH dose-response curve. Another limitation to this study is that we did not measure plasma AMPH levels, leaving the possibility that estradiol altered the pharmacokinetic profile of AMPH. Lukas et al. [31] recently reported that women achieved higher plasma levels of cocaine after intranasal administration during the follicular phase as compared with the luteal phase and attributed this difference to alterations in cocaine pharmacokinetics. However, in the present study, estradiol specifically affected some but not other effects of AMPH, making it unlikely that the interaction was related to absorption, distribution, or clearance.

In summary, these results provide limited evidence that the stimulating effects of AMPH are increased by acute estradiol pretreatment. This is consistent both with preclinical data and with previous data from our laboratory using human subjects. Future research in which dose and timing of estradiol administration are altered is necessary to determine the mechanism by which estradiol enhances the stimulating effects of AMPH.

Acknowledgments

This work was supported by grants from the NIH (R01 DA02812 and M01 RR00055). The authors thank Christopher O'Connor, Aaron York, Paul Meyer, and Angella Charnot for their excellent technical assistance.

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