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Effect of Menstrual Cycle Phase on Neuroendocrine and Behavioral Responses to the Serotonin Agonist *m*-Chlorophenylpiperazine in Women with Premenstrual Syndrome and Controls*

TUNG-PING SU, PETER J. SCHMIDT, MERRY DANACEAU, DENNIS L. MURPHY, AND DAVID R. RUBINOW

Behavioral Endocrinology Branch (T.-P.S., P.J.S., D.R.R.), Clinical Center Nursing Department (M.D.), and Laboratory of Clinical Science (D.L.M.), National Institute of Mental Health, Bethesda, Maryland 20892-1276

ABSTRACT

To evaluate the potential role of serotonin in the premenstrual syndrome (PMS), we investigated the effects of menstrual cycle phase on neuroendocrine and behavioral responses to the serotonergic agent *m*-chlorophenylpiperazine (m-CPP) in women with PMS and controls.

A single oral dose of m-CPP (0.5 mg/kg) was administered to 10 PMS patients and 10 healthy controls during the follicular and luteal phases of the menstrual cycle. We observed the following. m-CPP administration during the luteal phase resulted in an acute improvement of PMS symptoms; the plasma cortisol and ACTH responses to m-CPP were blunted in both menstrual cycle phases in PMS patients compared with controls.

These data provide evidence for the acute efficacy of m-CPP in the treatment of PMS. Although there is additional evidence for dysregulation of either the hypothalamic-pituitary-adrenal axis or serotonin control of the hypothalamic-pituitary-adrenal axis in women with PMS, there is little evidence for luteal phase-specific serotonergic dysfunction. These findings, nonetheless, implicate the serotonin system as a modulating (not causal) factor in PMS. (*J Clin Endocrinol Metab* **82:** 1220–1228, 1997)

PREMENSTRUAL syndrome (PMS) consists of a heterogeneous group of affective and somatic symptoms that occur during the luteal phase of the menstrual cycle. The potential role of the serotonin (5-HT) system in PMS is suggested by the following: 1) the role of 5-HT in many of the symptoms and behaviors characteristic of PMS (1–5); 2) diagnosis-related differences (between PMS patients and controls) in peripheral 5-HT measures (6–8) and neuroendocrine responses to 5-HT in some (9–11), but not all, studies (12); and 3) successful treatment of PMS with 5-HT agents (*e.g.* clomipramine, buspirone, and fluoxetine).

m-Chlorophenylpiperazine (m-CPP) has been used extensively as a probe for testing serotonergic function in several psychiatric disorders (13–22). Diagnosis-related alterations in the neuroendocrine responses to m-CPP and m-CPP-related alterations in symptomatology suggest an association between central 5-HT system function and the pathophysiology of these disorders. To help assess the potential role of 5-HT in PMS, we evaluated the behavioral and neuroendocrine responses to oral m-CPP administration in women with PMS and controls during the follicular and luteal phases of the menstrual cycle.

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Address all correspondence and requests for reprints to: David R. Rubinow, M.D., Behavioral Endocrinology Branch, National Institute of Mental Health, Building 10, Room 3N238, 10 Center Drive, MSC 1276, Bethesda, Maryland 20892-1276.

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Subjects and Methods

Subject selection

Women with PMS were either self-referred in response to advertisements in the local newspapers and hospital newsletter or were referred by their physicians. All women were screened for the absence of significant medical illness (current or in the past 2 yr) through history, physical examination, and routine laboratory tests (including thyroid function tests). All subjects were administered a structured psychiatric interview, the Structured Clinical Interview for DSM-III-R (23), to confirm the absence of significant current or recent (past 2 yr) axis I psychiatric illness, including alcohol and substance abuse. A positive distant past history (>2 yr before study entry) of axis I psychiatric illness was not an exclusion criterion for the women with PMS. Except for one woman who was on a stable regimen of thyroid hormone replacement for hypothyroidism, none of the women had taken psychoactive medications, hormonal preparations (including oral contraceptives), mineral supplements, or nonsteroid antiinflammatory medications within the past 6 months. All women reported having regular menstrual cycles, ranging from 23–33 days in length. Before participating in the study, each woman had the diagnosis of PMS prospectively confirmed by daily three-item visual analog scale self-ratings for 3 months. Each participant had at least a 30% increase in mean negative mood (*e.g.* depression, anxiety, or irritability) symptoms, relative to the actual range of the analog scale used, in the week before menses compared with those in the week after menses in at least two of three cycles, as described previously (24, 25). An exclusion criterion was the appearance of significant mood symptoms (mean mood ratings below the midpoint of the rating scale) during the follicular phase of the menstrual cycle. Approximately 30–40% of the women presenting to our clinic with symptoms of PMS met these diagnostic criteria. We retrospectively examined the records of the women participating in the study and found that all of those selected for participation also met the criteria for premenstrual dysphoric disorder of the Diagnostic and Statistical Manual of Mental Disorders (26). Pregnancy tests were performed before the beginning of the study, and all participants were required to employ barrier contraceptive methods to prevent pregnancy during the course of the study.

The protocol was reviewed and approved by the NIMH intramural research review board, and oral and written informed consents were obtained from all subjects.

The control group consisted of women (n = 10) without symptoms of PMS who had the absence of significant PMS symptoms confirmed in a manner similar to the patient group. Additionally, none had a past or current axis I psychiatric illness or the presence of significant medical illness.

Procedure

After acceptance into this study, all women were requested to undergo two oral m-CPP challenge tests during two consecutive menstrual cycle phases: luteal (the week before anticipated menses) and follicular (3–7 days after the end of menses) phases. m-CPP tests were administered at least a week apart. The order of m-CPP test performance during the luteal or follicular phase of the menstrual cycle was randomized. The season (*e.g.* winter *vs.* summer) during which m-CPP tests were performed was not randomized in either subject group.

The procedure for the m-CPP test [detailed previously (27)] consists of an open label oral administration of 0.5 mg/kg BW m-CPP at 1000 h (0 min) at least 45 min after iv catheter placement. Baseline blood samples and behavioral assessments were obtained at −15 and 0 min. During the next 3.5 h, plasma sampling (every 15 min) and behavioral self-ratings (every 30 min) were performed. These selected time points were based upon the results of previous m-CPP studies (27) indicating that peak neuroendocrine and behavioral responses occurred approximately 90–150 min after oral m-CPP administration.

Hormonal measures

Blood samples were collected for the measurement of plasma levels of cortisol, PRL, ACTH, GH, and m-CPP. The hormones measured were those previously shown to be stimulated by m-CPP. Plasma was stored at −70 C until the time of analysis. Plasma hormone assays were performed at Hazelton Laboratories (Vienna, VA) by methods previously described [cortisol (28), ACTH (29), GH (30), PRL (31), and progesterone (32)]. The intra- and interassay coefficients of variation were 3.8% and 10.7% for cortisol, 8.5% and 20.3% for ACTH, 2.1% and 6.4% for GH, 4.6% and 8.2% for PRL, and 6.1% and 9.1% for progesterone, respectively. Plasma m-CPP levels were measured as described previously (27).

Symptom ratings

Symptom self-ratings included the following. 1) The NIMH Self-Rating Scale (27) is a seven-point scale measuring the severity of six symptom categories, including anxiety, depressive affect, dysphoria (unpleasant mood), activation-euphoria, altered self-reality, and impaired function. Each symptom category comprised several symptoms (27). 2) The Visual Analog Scale (VAS) is a 100-mm line measuring the severity of symptoms commonly associated with PMS. A composite mood symptom score was calculated by averaging the four mood symptom scores commonly experienced by women with PMS (sadness, anxiety, irritability, and mood lability). 3) The Rating Scales for Premenstrual Tension Syndrome (33), both self (PMTS-Self) and observer (PMTS-Rater) forms, were administered at baseline before m-CPP administration. The PMTS-Rater scale was also employed 180 min after m-CPP administration.

Statistical analysis

Neuroendocrine responses and m-CPP plasma levels. Plasma hormone and m-CPP levels obtained at baseline and 30, 60, 90, 120, 150, 180, and 210 min after m-CPP were compared by ANOVA with repeated measures (ANOVA-R; Systat, SPSS, Chicago, IL), with diagnostic group as the between-subject variable and menstrual cycle phase and time point as the within-subject variables. Additionally, measures of plasma hormone secretion, including baseline levels, integrated area under the secretory curve (AUC; calculated by the trapezoidal integration method), maximum plasma level during the m-CPP test (MAX), and the difference between baseline level and maximum level (Δ max) were compared by ANOVA-R with diagnostic group (PMS and controls) as the between-subject variable and menstrual cycle phase (luteal and follicular) as the within-subject variable. Significant differences in plasma hormone and m-CPP levels were analyzed by *post-hoc* Bonferroni *t* tests. Values are reported as the mean ± SD.

Behavioral ratings. Behavioral self-ratings during the m-CPP challenge were analyzed by ANOVA-R with diagnostic group (PMS and controls) as the between-subject variable and menstrual cycle phase (luteal and follicular) and time point (baseline to 210 min) as the within-subject variables. Significant differences in these measures were analyzed with *post-hoc* Bonferroni *t* tests.

Additional analyses. Neuroendocrine measures and behavioral ratings were reanalyzed in a manner similar to that described above, with the exclusion of those women (n = 2) with PMS who were not symptomatic (PMTS-Self score, <4) during the luteal phase m-CPP challenge. ANOVA-R was also used to examine possible order effects (*i.e.* order of menstrual cycle phases during which m-CPP tests were performed) in the neuroendocrine and behavioral responses to m-CPP. Finally, the neuroendocrine responses to m-CPP were reanalyzed by ANOVA-R with age as the covariate.

Results

Subject characteristics

Fifteen women with PMS and 10 controls were enrolled in the m-CPP challenge test, and 10 women in each group completed both the luteal and follicular phase studies. Five women with PMS declined a second m-CPP test as a result of intolerable side-effects of m-CPP, specifically migraine-like headaches (follicular, n = 4; luteal, n = 1). The age range was from 28–44 yr in women with PMS and from 22–39 yr in controls. There was a trend ($P = 0.09$) for a significant difference in the mean (±SD) ages in women with PMS (35.1 ± 5.6 yr) compared to controls (30.8 ± 4.9 yr). Two of the 10 women with PMS completing both infusions were not symptomatic during the luteal phase m-CPP test (baseline PMTS-Rater, <4).

A structured diagnostic interview identified 6 of 10 patients completing 2 m-CPP tests who met criteria for a past history of affective disorder. There were no significant differences in the past psychiatric history between the 10 women with PMS studied in both phases of the cycle and those completing only 1 study (by Fisher exact test, $P = 0.09$).

Test days and menstrual cycle characteristics

m-CPP challenge tests were performed in the follicular phase between days 3–9 (day 6.0 ± 1.8 and day 6.6 ± 1.7 for PMS and controls, respectively) and in the luteal phase between days −2 and −12 (day −5.5 ± 2.8 and day −6.0 ± 2.9 for PMS and controls, respectively). Plasma levels of progesterone during the luteal phase testing were greater than 6 nmol/L in all subjects, suggesting the presence of ovulatory menstrual cycles.

Plasma m-CPP levels

ANOVA-R of the plasma levels of m-CPP identified a uniform increase in plasma levels of m-CPP after m-CPP administration in both patients and controls that did not differ between the follicular and luteal phases of the menstrual cycle. Mean plasma m-CPP levels were, in general, higher in controls than in patients, reflecting a greater variability in plasma m-CPP levels in the control group. However, ANOVA-R of the plasma levels of m-CPP identified no

significant effects of diagnosis ($F_{1,17} = 0.8$; P = NS); diagnosis and menstrual cycle phase interactions ($F_{1,17} = 0.8$; P = NS); or diagnosis, menstrual cycle phase, and time interactions ($F_{7,119} = 0.7$; P = NS).

Plasma hormone levels (Table 1 and Figs. 1 and 2)

Cortisol. ANOVA-R demonstrated significant effects of diagnosis, menstrual cycle phase, and diagnosis by phase interactions on post-m-CPP plasma cortisol levels. Compared to controls, women with PMS had significantly lower plasma cortisol levels (mean of individual time points 0–210 min) during the luteal phase and slightly, but not significantly, lower levels during the follicular phase.

ANOVA-R demonstrated a significant diagnosis effect on the maximum plasma cortisol level (MAX) and a trend for significance of the effect of diagnosis in the AUC values of plasma cortisol, consistent with the lower post-m-CPP plasma cortisol levels in women with PMS compared to controls. No other significant effects were observed.

ACTH. ANOVA-R showed significant effects of time and time by diagnosis on m-CPP-stimulated ACTH levels, reflecting reduced plasma ACTH levels in women with PMS compared to controls. No significant effects of phase, diagnosis by phase, or diagnosis by phase by time were observed.

ANOVA-R revealed a trend for a significant effect of diagnosis on baseline plasma ACTH levels and for an effect of diagnosis on AUC, reflecting higher baseline ACTH levels but less total ACTH release after m-CPP administration in women with PMS compared to controls. ANOVA-R did not identify significant differences between patients and controls or menstrual cycle phase differences in other measures of ACTH secretion.

PRL. ANOVA-R identified no diagnosis effect, but did find a significant time effect and a trend for a menstrual cycle phase effect on the m-CPP-stimulated PRL levels, reflecting a modest, but nonsignificant, increase in m-CPP-stimulated plasma PRL levels during the luteal compared to the follicular phase in both women with PMS and controls.

ANOVA-R identified a trend for a menstrual cycle phase effect on AUC and a significant phase effect on MAX and Δ max, reflecting a somewhat greater release of PRL by m-CPP in the luteal than in the follicular phase in both groups of women.

ANOVA-R also identified a trend for a diagnosis effect on

TABLE 1. Basal and m-CPP-stimulated plasma hormone levels

Variable	Cycle phase	Control [Mean (SD)]	PMS [Mean (SD)]	Diagnosis $F_{1,17}$	Phase $F_{1,17}$	Diagnosis × Phase $F_{1,17}$
Cortisol (nmol/L)						
Baseline	Pre	284 (99)	246 (110)	0.3	3.2^a	0.7
	Post	223 (63)	223 (99)			
AUC	Pre	30,046 (28,694)	10,981 (24,500)	2.4^a	0.4	1.4
	Post	25,521 (24,831)	14,623 (17,354)			
MAX	Pre	651 (157)	439 (138)	$5.6^{b,c}$	1.1	1.8
	Post	546 (248)	452 (141)			
Δmax	Pre	367 (193)	193 (155)	4.1^a	0.0	0.9
	Post	326 (232)	229 (110)			
ACTH (pmol/L)						
Baseline	Pre	5.1 (1.8)	5.3 (1.8)	2.8^a	0.8	1.9
	Post	4.5 (0.9)	8.2 (7.3)			
AUC	Pre	537 (386)	242 (204)	4.1^a	0.3	1.1
	Post	638 (797)	−161 (1,253)			
MAX	Pre	13.2 (6.6)	9.5 (3.9)	0.1	0.1	2.8^a
	Post	10.3 (5.7)	12.8 (7.1)			
Δmax	Pre	8.1 (6.8)	4.2 (3.9)	2.2	0.3	0.6
	Post	5.9 (6.4)	4.5 (4.5)			
PRL (μg/L)						
Baseline	Pre	4.7 (4.1)	14.3 (21.3)	1.4	1.0	2.0
	Post	9.2 (5.2)	13.6 (11.2)			
AUC	Pre	2,969 (2,043)	1,583 (1,478)	2.8^a	2.9^a	0.5
	Post	1,912 (3,572)	690 (686)			
MAX	Pre	40.6 (19.6)	32.1 (18.7)	0.5	$4.1^{b,d}$	0.2
	Post	29.6 (29.7)	25.2 (20.0)			
Δmax	Pre	35.9 (20.6)	17.8 (13.2)	3.8^a	$4.5^{b,e}$	0.8
	Post	20.6 (28.7)	11.6 (6.5)			
GH (ng/ml)						
Baseline	Pre	1.3 (2.0)	1.2 (1.4)	0.1	0.2	0.3
	Post	1.3 (2.2)	1.0 (1.5)			
AUC	Pre	60 (342)	386 (687)	0.9	1.7	1.4
	Post	37 (597)	176 (573)			
MAX	Pre	3.8 (3.0)	6.2 (6.2)	0.5	0.8	2.7^a
	Post	4.2 (3.9)	4.8 (6.1)			
Δmax	Pre	2.5 (2.9)	5.1 (6.0)	0.6	0.5	2.0
	Post	2.9 (4.1)	3.9 (6.4)			

By ANOVA-R: P = NS for all F values listed, except $^aP \leq 0.1$; $^bP \leq 0.05$. By Bonferroni t comparisons: PMS *vs.* controls: $^cP \leq 0.05$. Follicular *vs.* luteal: $^dP \leq 0.1$; $^eP \leq 0.05$.

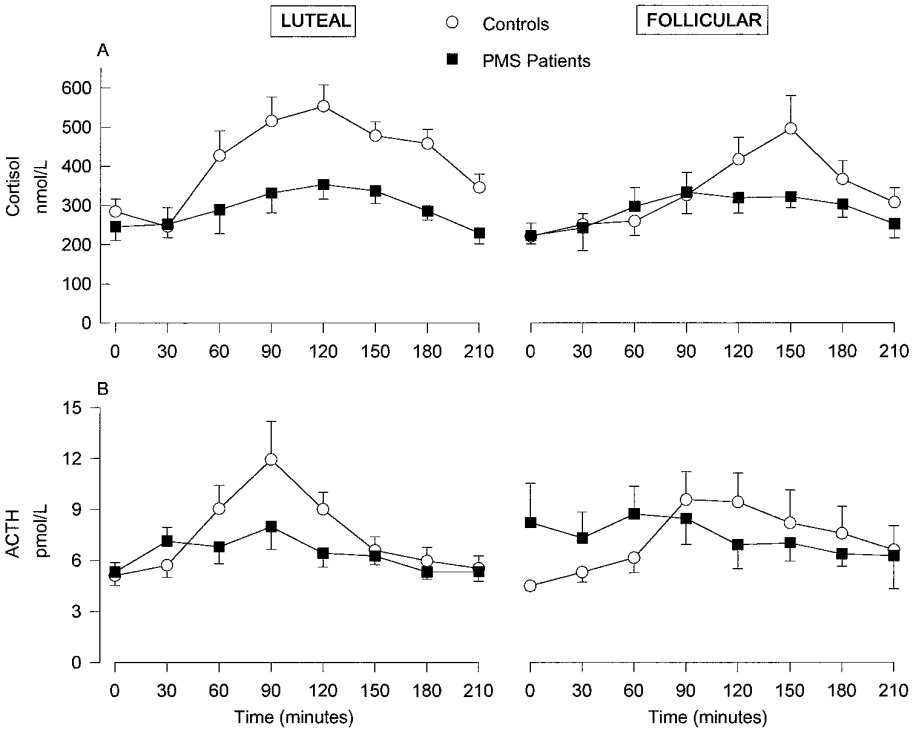

FIG. 1. A, Significant effects of time ($F_{7,119} = 8.9$; $P < 0.01$), diagnosis ($F_{1,17} = 5.8$; $P < 0.05$), menstrual cycle phase ($F_{1,17} = 8.2$; $P < 0.01$), and diagnosis by phase interaction ($F_{1,17} = 6.6$; $P < 0.05$) were observed after m-CPP administration on plasma cortisol levels. The significant menstrual cycle phase effect disappeared when plasma cortisol levels were reanalyzed by ANOVA-R with age as a covariate. Significant *post-hoc* Bonferroni *t* tests of plasma cortisol levels (mean of both phase values at each time point), compared to baseline, showed increases at 60–180 min ($t_{126} = 3.4$–7.2; $P < 0.01$) in both patients and controls; significantly lower cortisol levels were observed across both menstrual cycle phases in women with PMS compared with controls ($t_{17} = 2.4$; $P < 0.05$). The diagnosis by cycle phase effect is consistent with significantly higher plasma cortisol levels (mean of individual time points 0–210 min) in controls compared with women with PMS during the luteal phase m-CPP challenge ($t_{34} = 3.2$; $P < 0.01$). B, A trend effect of diagnosis ($F_{1,17} = 4.1$; $P = 0.06$) and significant effects of time and time by diagnosis ($F_{7,119} = 5.2$ and 2.3, respectively; $P < 0.05$) were observed after m-CPP administration on plasma ACTH levels, reflecting blunted ACTH responses across both menstrual cycle phases in women with PMS compared with controls. *Post-hoc* Bonferroni *t* tests identified significant increases in plasma ACTH levels (mean of both phase values at each time point), compared to baseline values, at 90 and 120 min ($t_{136} = 4.1$ and 2.9; $P < 0.01$ and 0.05, respectively) in controls, but not in women with PMS at any time point. There was a trend effect of diagnosis on baseline plasma ACTH levels ($F_{1,17} = 2.8$; $P = 0.1$), consistent with the significant *post-hoc* test finding that higher baseline ACTH levels were observed (primarily in the follicular phase) in PMS patients compared with controls ($t_{18} = 2.5$; $P < 0.05$).

AUC of PRL levels, which disappeared when covaried for age. There were no differences in AUC plasma PRL levels between patients and controls in either the luteal or follicular phase m-CPP test.

GH. ANOVA-R identified a significant effect of time on GH secretion after m-CPP administration in patients and controls.

There were no significant effects of diagnosis, menstrual cycle phase, or diagnosis by phase interactions on AUC, MAX, and Δ max GH levels.

Additional analyses. Baseline plasma levels of cortisol, PRL, and GH were not significantly different between patients and controls or across menstrual cycle phases (Table 1). Additionally, a reanalysis, excluding the two women with PMS who were not symptomatic during the luteal phase testing, did not alter the results of the original analyses of m-CPP stimulated cortisol, ACTH, PRL, and GH release. ANOVA-R revealed no significant order effects, *i.e.* results were independent of the menstrual cycle phase in which m-CPP was first administered. Finally, as m-CPP levels were nonsignificantly reduced in patients compared with controls, ANOVA-Rs showing significant group effects (cortisol and ACTH) were reperformed with m-CPP AUCs as the changing covariates. The original observations were not altered by this analysis of covariance.

Behavioral ratings

Some of the results of the behavioral effects of m-CPP administration in the women with PMS and controls from this study will be reported elsewhere (Su, T.-P., Schmidt PJ, Danaceau MA, Tobin MB, Rosenstein DL, Murphy DL, Rubinow OR, unpublished manuscript).

NIMH self-rating subscales. ANOVA-R comparisons of the baseline scores of the subscale symptoms of depression, dysphoria, anxiety, and feelings of impaired function identified significant effects of diagnosis ($F_{1,18} = 4.5$–16.2; $P < 0.05$), menstrual cycle phase ($F_{1,18} = 5.2$–17.1; $P < 0.05$), and diagnosis by phase interaction ($F_{1,18} = 5.2$–17.1; $P < 0.05$), which reflected significant increases in the severity of mood and behavioral symptoms at baseline in the women with PMS compared with controls during the luteal phase ($t_{36} = 3.6$–5.7; $P < 0.01$). No significant differences were observed

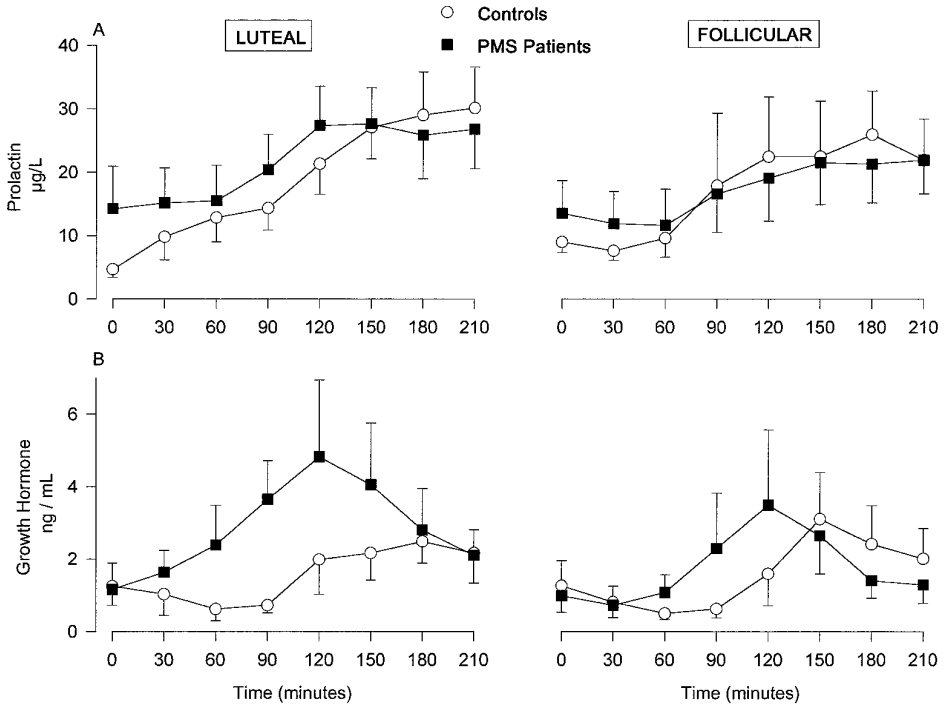

FIG. 2. A, A significant effect of time ($F_{7,119} = 14.6$; $P < 0.001$) and a trend for a menstrual cycle phase effect ($F_{1,17} = 3.1$; $P < 0.1$) were observed after m-CPP administration on plasma PRL levels, reflecting slight, but nonsignificant, increases in PRL levels during the luteal compared to the follicular phase in both PMS patients and controls. Significant *post-hoc* Bonferroni *t* tests of plasma PRL levels (mean of both phase values at each time point) compared to the levels at baseline showed increases at 90 min ($t_{126} = 3.5$; $P < 0.05$) and 120–210 min ($t_{126} = 5.1$–6.1; $P < 0.01$). B, Similarly, a significant effect of time was observed after m-CPP administration on GH levels ($F_{7,119} = 2.3$; $P < 0.05$), consistent with significant increases in plasma GH levels compared to baseline values at 120 and 150 min (both $t_{126} = 2.7$; $P < 0.05$).

between patients and controls in the baseline scores of the two other NIMH subscale scores, *i.e.* activation/euphoria and altered self-reality.

ANOVA-R comparing the symptom response to m-CPP demonstrated a significant interaction effect of diagnosis, menstrual cycle phase, and time on the subscale self-ratings of dysphoria and depression, reflecting significant decreases in the severity of dysphoria and depression after m-CPP administration in women with PMS, but not in controls, during the luteal phase (Fig. 3). Consistent with the observed improvement in the symptoms of dysphoria and depression, ANOVA-R showed a similarly significant diagnosis by menstrual cycle phase by time effect on the activation-euphoria subscale, reflecting a significant increase in the feeling of well-being during the luteal phase m-CPP test in women with PMS but not controls.

ANOVA-R did not identify significant effects of m-CPP administration on the scores of other NIMH subscales, with the exception of functional impairment, reflecting an increase in problems concentrating in the patients after m-CPP during both phases of the menstrual cycle.

VAS composite mood ratings. ANOVA-R showed significant effects of diagnosis, menstrual cycle phase, phase by diagnosis interactions, time, and time by diagnosis interactions on the composite PMS mood symptom ratings (see Fig. 4).

PMTS-Rater scores. Consistent with the significant diagnosis by phase interaction observed for baseline PMTS-Rater scores ($F_{1,12} = 30.1$; $P < 0.001$), women with PMS had a significant increase in baseline scores during the luteal phase compared with the follicular phase ($t_{36} = 7.9$; $P < 0.01$) and compared to the luteal phase scores in controls ($t_{36} = 8.6$; $P < 0.01$). ANOVA-R showed a significant interaction of menstrual cycle phase with time and diagnosis ($F_{1,18} = 20.7$; $P < 0.001$) on PMTS-Rater scores, reflecting a significant decrease (69%) from the baseline rating at 180 min during the luteal m-CPP test in women with PMS ($t_{36} = 6.0$; $P < 0.01$). This change in PMTS-Rater scores was not observed during the follicular phase m-CPP challenge in women with PMS or in the control group during either challenge.

Discussion

Our data suggest that m-CPP administration during the luteal phase of the menstrual cycle resulted in an acute improvement of the symptoms of PMS. Additionally, our data identified differences in the neuroendocrine response to m-CPP in women with PMS compared to controls including the following: a decreased plasma cortisol response to m-CPP in both phases of the menstrual cycle, which reached statistical significance only during the luteal phase of the cycle; and a reduced plasma ACTH response to m-CPP in both phases of the menstrual cycle. In addition, we observed, in both women with PMS and controls, menstrual cycle phase-related differences in some physiological responses to m-CPP administration, including increased cortisol secretion and a trend for an increased PRL secretory AUC during the luteal compared to the follicular phase of the menstrual cycle. We

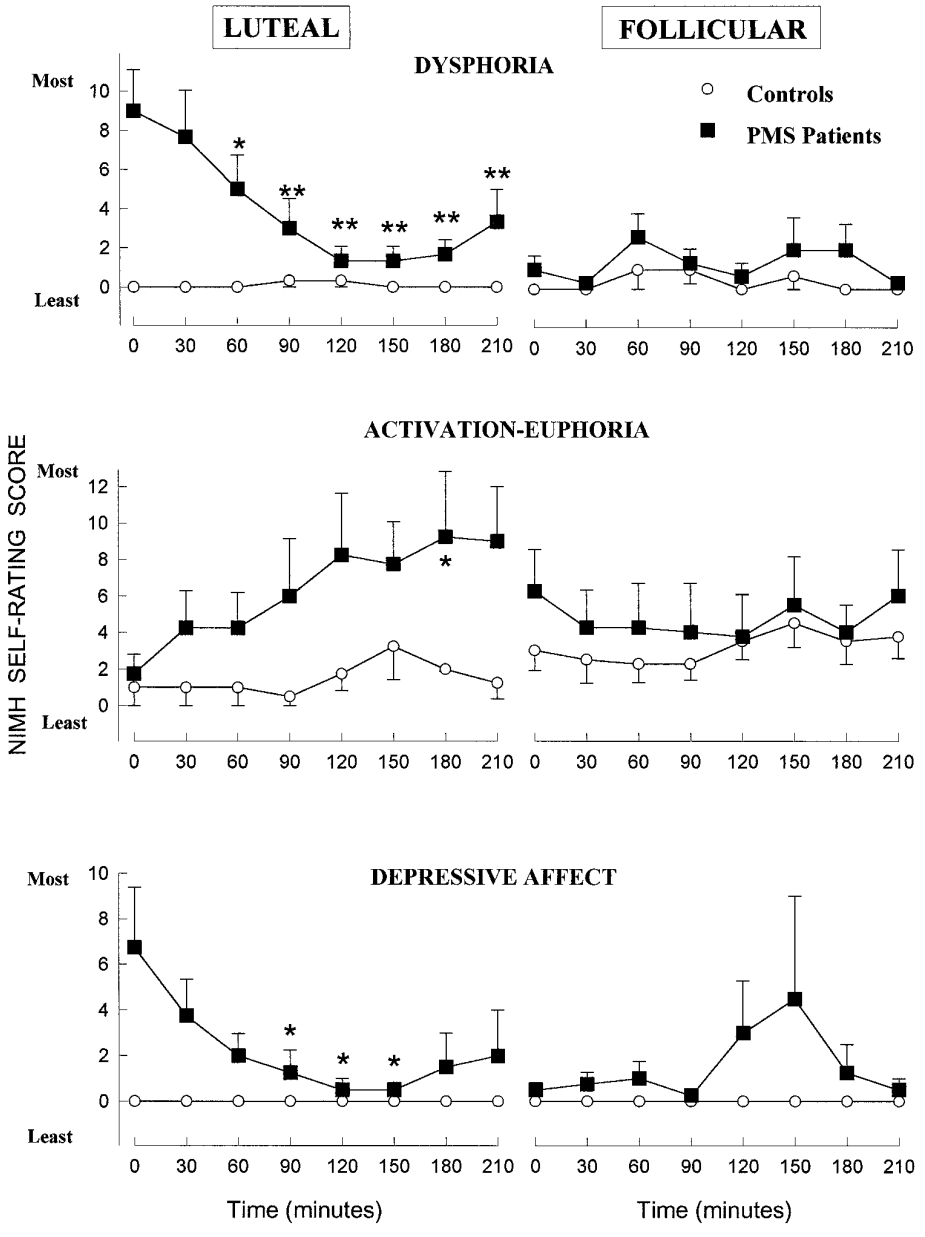

FIG. 3. Significant interaction effects of diagnosis, menstrual cycle phase, and time were observed during m-CPP challenge on NIMH dysphoria, depression, and euphoria subscale ratings ($F_{7,126}$ = 4.2, 2.1, and 2.5, respectively; $P < 0.05$); *i.e.* luteal phase m-CPP administration resulted in significant decreases in dysphoria and depression and increases in feelings of well-being in women with PMS, but not in controls. Significant *post-hoc* Bonferroni *t* tests of symptom ratings compared with baseline ratings are indicated as follows: **, $P < 0.01$ (t_{144} = 4.0–5.4); *, $P < 0.05$ (t_{144} = 2.7–3.2).

found no changes in measures of the GH response to m-CPP administration related to either diagnostic category or menstrual cycle phase.

m-CPP administration has been reported to induce anxiety as well as activation and euphoria in both healthy normal controls and subjects with a variety of neuropsychiatric conditions (13, 14, 17, 19, 27, 34–39). In several neuropsychiatric disorders (*e.g.* panic disorder and obsessive-compulsive disorder), m-CPP is associated with symptom exacerbation. Similarly, one uncontrolled study of m-CPP administration (0.5 mg/kg, orally) in women with PMS (sample size not reported) (40) suggested that m-CPP administration resulted in an increase in dysphoric symptoms. In contrast, we observed a uniform improvement after m-CPP in the symptoms of depression, irritability, and anxiety in women with PMS during the luteal phase (regardless of the scales employed). Neither the asymptomatic controls nor the women with PMS who were not symptomatic during the luteal phase testing reported an increase in symptoms of dysphoria or feelings of well-being, suggesting, therefore, that symptom improvement after m-CPP in women with PMS is not consequent to nonspecific mood enhancement by m-CPP. The relatively acute onset of the salutary effects of serotonergic stimulation with either m-CPP (hours) or medications such as fluoxetine and clomipramine (days to weeks) suggests that the mechanism of action of these agents in PMS is not a classical antidepressant effect, which usually requires 3–6 weeks before becoming clinically meaningful. Similar mood-enhancing effects after m-CPP administration have been reported in subjects with seasonal affective disorder, a condition resembling PMS in the atypical pattern of depressive symptoms (41) present in both conditions. In contrast, m-CPP admin-

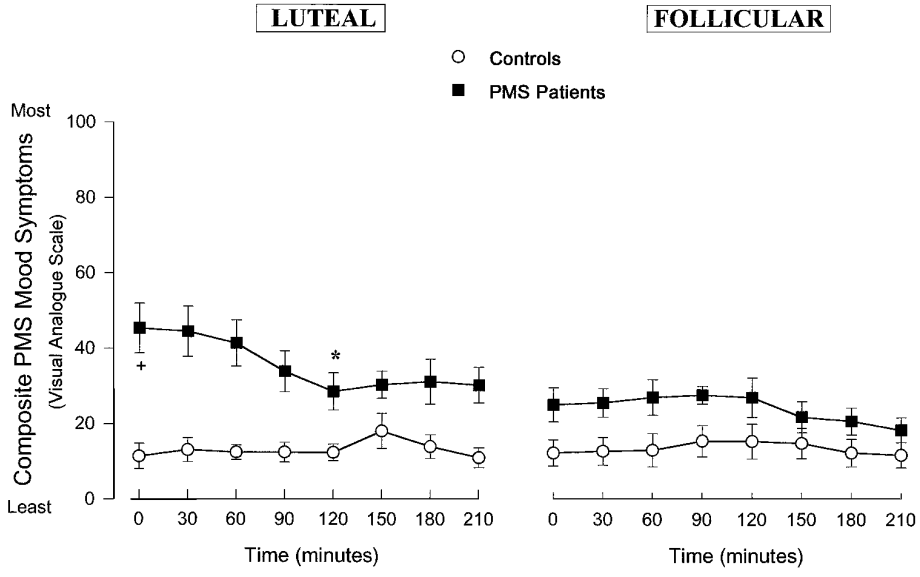

FIG. 4. ANOVA-R revealed significant interactions of menstrual cycle phase with diagnosis ($F_{1,18} = 6.7$; $P < 0.05$) on baseline composite mood symptoms (sadness, anxiety, mood lability, and irritability), indicating significantly more severe mood symptoms at baseline during the luteal phase in women with PMS compared to controls (Bonferroni $t_{36} = 5.2$; +, $P < 0.01$). Further, significant effects of diagnosis ($P < 0.005$), menstrual cycle phase ($P < 0.05$), phase by diagnosis interaction ($P < 0.05$), time ($P < 0.05$), and time by diagnosis interaction ($P < 0.01$) were observed after m-CPP administration on VAS composite mood symptom scores. The time by diagnosis effect is consistent with the observed decrease (compared to baseline) in symptom scores in PMS patients during the luteal phase after m-CPP ($t_{144} = 2.8$; *, $P < 0.05$).

istration has symptom-provoking effects in anxiety disorders such as panic disorder (16, 42) and obsessive-compulsive disorder (13, 14), with an absence of behavioral effect reported in major depression (18, 43).

Baseline plasma cortisol levels were not different between patients and controls in either phase of the menstrual cycle, consistent with earlier basal hormone studies (44). However, compared to controls, we observed a reduction in the stimulated plasma cortisol levels in women with PMS in both phases of the menstrual cycle, which only reached statistical significance during the luteal phase. Similarly, diagnosis-related differences in the cortisol secretory AUC, the maximum stimulated plasma cortisol value, and the Δ max (difference between maximum stimulated and baseline values) suggest either a decreased HPA axis responsivity to 5-HT stimulation or less effective 5-HT stimulation in women with PMS. Consistent with our data, a previous report by Bancroft *et al.* (10) reported a blunting of the plasma cortisol response to L-tryptophan administration in both phases of the menstrual cycle in women with perimenstrual mood changes compared to controls. In contrast, Rabin *et al.* (45) observed an enhanced cortisol response to CRH in women with PMS compared to controls. It appears, therefore, that the HPA axis in women with PMS is not uniformly hyporesponsive, but is less responsive to 5-HT stimulation [although diagnosis-related differences in 5-HTP-stimulated cortisol were not observed by Veeninga *et al.* (12)].

Consistent with this suggestion is the observation of a blunted plasma ACTH response to m-CPP in patients compared to controls. This blunting appeared in both menstrual cycle phases, accompanied in the follicular phase by elevated baseline ACTH levels relative to control values. No previous studies in women with PMS have reported ACTH levels in response to 5-HT probes. It is of interest that our observation of a blunted ACTH response to m-CPP in women with PMS is again paralleled by a similar blunting in the ACTH response to m-CPP observed in patients with seasonal affective disorder (Schwartz PJ, Murphy DL, Wehr TA, Garcia-Borreguero D, Oren DA, Moul DE, Ozaki N, Shelbaker AJ, Rosenthal NE, unpublished manuscript).

Similar to the findings of Bancroft *et al.* (10), but in contrast to those of Yatham (46), we identified no diagnosis-related differences in the PRL response to m-CPP in women with PMS. Additionally, Bancroft *et al.* observed that both women with PMS and controls had a relative blunting of L-tryptophan-induced PRL secretion during the luteal compared to the follicular phase. In contrast, we observed a trend for the PRL response to m-CPP to be enhanced (and the Δ max PRL to be significantly increased) during the luteal compared to the follicular phase, consistent with the report by Dinan *et al.* (11) of an enhanced PRL response to the 5-HT_{1A} agonist buspirone during the luteal compared to the follicular phase in asymptomatic women. Data from O'Keane *et al.* suggest that the differences in the patterns of PRL response observed in this study and that of Bancroft *et al.* (10) may be related to different timing of samples (and, thus, estradiol levels) during the follicular phase.

Several additional factors have been reported to potentially influence serotonergically mediated stimulation of PRL and cortisol secretion, including season and plasma m-CPP levels. First, Brewerton *et al.* (47) reported that L-tryptophan- and m-CPP-stimulated PRL levels were enhanced in the fall and winter compared to those in the summer, although lower baseline PRL levels during the winter have also been observed (48). Previous studies have also consistently shown a seasonal increase (during winter) in baseline cortisol levels (49). In our study, eight controls and only four women with PMS had m-CPP tests performed in the winter. Hence, the

slightly, but not significantly, elevated baseline PRL levels in women with PMS may reflect the fact that fewer women with PMS received m-CPP in the winter months compared to the controls. We did not, however, observe a significant difference in baseline cortisol in patients and controls despite the seasonal differences in the timing of m-CPP tests. Second, maximum m-CPP plasma levels correlated with the AUC of cortisol, ACTH (luteal phase testing only), and PRL in subjects with PMS and controls. However, no differences in the maximum m-CPP levels were observed between patients and controls, and it is unlikely, therefore, that plasma m-CPP levels account for the observed differences in the neuroendocrine response to m-CPP in patients *vs.* controls.

We observed no diagnosis-related differences in the pattern of GH response to m-CPP administration. This raises several issues. First, a stimulated GH response to oral m-CPP has not been previously reported (50), but has been seen after iv m-CPP, with values comparable to those that we obtained. Second, our failure to observe diagnosis-related differences contrasts with the findings of Bancroft *et al.* (10) of a blunted GH response to L-tryptophan during both the follicular and luteal phases of the menstrual cycle in women with PMS compared to controls. Baseline plasma levels of GH, which may vary considerably and potentially alter the pattern of GH response to a particular challenge agent, did not differ between women with PMS and controls, nor did they differ across menstrual cycle phases, in contrast to the menstrual cycle-related effects on GH reported by O'Keane *et al.* (51). In fact, the baseline levels of plasma GH in this study were comparable to those reported by Bancroft *et al.* (10). Given that blunting of GH response to iv m-CPP has been reported in persons with major depression (18), the sample of women studied by Bancroft *et al.* (10) may have had a higher prevalence of women with depressive illness. Alternatively, oral m-CPP may not as reliably stimulate GH as does L-tryptophan.

m-CPP has a moderately high affinity for a number of 5-HT receptors (particularly 5-HT_{2C}, 5-HT_{2A}, 5-HT_3, and 5-HT_{1A}) as well as a moderate affinity for α_2-adrenergic receptors and a somewhat lower affinity for other adrenergic and dopaminergic receptors (52, 53). Studies with 5-HT receptor antagonists have led to the attribution of most of the neuroendocrine and behavioral effects of m-CPP to its partial agonist effects on the 5-HT_{2c} receptors and antagonist effects on the 5-HT_{2A} receptors (54). However, as reviewed by Murphy *et al.* (55), the general lack of concordance among different endpoint responses to m-CPP (particularly neuroendocrine *vs.* behavioral measures) suggests that no simple relationship can be inferred between responses to m-CPP and central serotonergic function in general. Nonetheless, the consistent therapeutic efficacy of 5-HT agents in PMS strongly suggests that the 5-HT system is involved in the pathophysiology of PMS symptoms. Furthermore, the findings of this present study suggest possible specific dysregulations of the 5-HT_{2A} and 5-HT_{2C} subsystems to be present in women with PMS.

In summary, this study provides further evidence of the salutary effects of acute serotonergic stimulation on mood and behavior in women with PMS. The observed mood and behavioral changes after m-CPP administration in women with PMS were not studied under double blind, placebo-controlled conditions and, thus, must be interpreted with caution. However, they are consistent with several double blind studies of clomipramine and fluoxetine that have reported a relatively rapid improvement in mood and behavioral symptoms in women with PMS. Further, we observed alterations of neuroendocrine function in women with PMS that may reflect either principle dysregulations of the HPA axis in women with PMS or, alternatively, are suggestive of dysregulation of the serotonergic control of the HPA axis. The complexities of serotonergic control of the physiological parameters measured in this study preclude inference about a direct relationship between serotonergic dysfunction and the pathophysiology of premenstrual syndrome. Furthermore, the majority of abnormalities in the neuroendocrine response to serotonergic agents in PMS have been observed during both phases of the cycle. Thus, possible serotonergic dysfunction is not suggested to be a direct cause of PMS, but may convey a vulnerability to mood destabilization in association with changes in gonadal steroids such as those seen during the course of the normal menstrual cycle. It is also possible that these abnormalities in the neuroendocrine response to serotonergic challenge may be predictive of response (or nonresponse) to treatment with serotonergic agents such as clomipramine or fluoxetine. Future studies should attempt to identify the mechanisms involved in the interaction between gonadal steroids and central serotonergic function that may be related to the symptoms in women with PMS.

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