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# Sensitivity to CCK-4 in Women with and without Premenstrual Dysphoric Disorder (PMDD) During Their Follicular and Luteal Phases

*Jean-Michel Le Mellédo, M.D., Salima Merani, Ph.D., Diana Koszycki, Ph.D., Francois Bellavance, Ph.D., Roberta Palmour, Ph.D., Jolanta Gutkowska, Ph.D., Susanne Steinberg, M.D., FRCPC, Daniel Georges Bichet, M.D., FRCPC, and Jacques Bradwejn, M.D., FRCPC*

*The authors determined whether women with premenstrual dysphoric disorder (PMDD) exhibit a heightened sensitivity to the panicogenic effects of CCK-4 administration and whether this enhanced sensitivity to CCK-4 would vary with the phase of the menstrual cycle at the time of CCK-4 injection. Twenty-one normal controls and 18 PMDD women were randomly assigned to receive the first and second CCK-4 injection during the follicular phase and the luteal phase or vice versa. PMDD women showed a greater anxiety and panic response to CCK-4. These preliminary results suggest that the CCK-B system may play a role in the pathophysiology of PMDD.*
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Premenstrual dysphoric disorder (PMDD) is a clinical syndrome characterized by significant mood, behavioral, and somatic symptoms. These symptoms regularly occur in the late luteal phase (LP) of the menstrual cycle, begin to remit after the onset of menstrual bleeding, and are typically absent in the week following menses. Anxiety seems to be a salient characteristic of the disorder (Hurt et al. 1992). Stein et al. (1989) reported that women with premenstrual disturbances who were free of any concurrent psychiatric disorder exhibited marked increases in self-rated anxiety in the premenstrual phase of the cycle. Other investigators have found that a substantial proportion of women diagnosed with severe premenstrual symptoms suffer from one or more anxiety disorders (Fava et al. 1992; Veeninga et al. 1994). The menstrual cycle (MC) also seems to contribute to the exacerbation of pathological anxiety in women suffering from panic disorder (Cameron et al. 1986; Cameron et al. 1988; Breier et al. 1986; Kaspi et al. 1994; Sanberg et al. 1986). McLeod et al. (1993) reported that women with both generalized anxiety disorder and PMDD experienced more severe anxiety symptoms than did women with generalized anxiety disorder alone. These findings suggest that the presence of PMDD may have an adverse effect on the clinical course of anxiety disorders.

Although the cause of PMDD remains unknown, the symptomatic overlap between anxiety and PMDD re-

From the Clarke Institute of Psychiatry and the Department of Psychiatry, University of Toronto, Toronto, Ontario; the University of Montreal, Montreal, Quebec; McGill University, St. Mary's Hospital, Montreal, Québec, Canada.

Address correspondence to: Dr. Jean-Michel Le Mellédo, Department of Psychiatry, University of Alberta, 1E7.26 Mackenzie Centre, Edmonton, Alberta, T6G 2B7, Canada.

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ported in studies raises questions as to shared underlying biological abnormalities. Available data to date seem to support this proposal. For example, pharmacological data indicate that alprazolam, a high-potency benzodiazepine agonist, and antidepressant agents that strongly inhibit serotonin reuptake (e.g., fluoxetine, clomipramine) are clinically effective in treating symptoms of both anxiety disorders (Ballenger et al. 1988; Gorman et al. 1987; Modigh et al. 1993) and PMDD (Harrison et al. 1990; Freeman et al. 1995; Steiner et al. 1995; Sandberg et al. 1993). As with anxiety disorder patients, women with PMDD also show a heightened sensitivity to panicogenic challenges. For instance, sodium lactate infusion (Fachinetti et al. 1992; Sandberg et al. 1993) and 35% $CO_2$ inhalation (Harrison et al. 1989) have been found to elicit significantly greater anxiety and clinical manifestations of panic in subjects with PMDD than in control women.

The main objective of the present study was to investigate the relationship between PMDD and anxiety further. We assessed the potential contribution of the cholecystokinin (CCK) system to the expression of anxiety in PMDD. The putative role of CCK in the expression of fear and anxiety has been confirmed by a number of animal and human studies (for review, see Bradwejn and Vasar 1995). Human studies have revealed that systemic administration of central CCK-B receptor agonists (i.e., CCK-4, pentagastrin) are profoundly panicogenic, provoking significant somatic, affective, and cognitive symptoms of panic as well as concomitant increases in heart rate and blood pressure (Bradwejn et al. 1991; Abelson and Nesse 1994). Furthermore, the effectiveness of these CCK-B agonists in provoking symptoms of panic anxiety is more evident in patients with panic disorder (Bradwejn et al. 1991; Abelson and Nesse 1994), generalized anxiety disorder (Brawman-Mintzer et al. 1996) and social phobia (McCann et al. 1995) than in healthy controls. Concentrations of CCK-8, a mixed CCK-A and -B receptor agonist, have been found to be lower in CSF and lymphocytes of panic disorder patients as compared to healthy volunteers (Lydiard et al. 1992; Brambilla et al. 1993). In light of evidence that suggests a link between PMDD and anxiety and between anxiety and CCK-4, we opted to determine whether PMDD women would also exhibit a heightened sensitivity to the panicogenic effects of the CCK receptor agonist, CCK-4.

We also decided to investigate whether a difference in sensitivity to CCK exists only during the LP or also during the follicular phase (FP). Such information could help elucidate whether a difference in sensitivity to a panicogenic agent is state- or trait-related. In the only study (Harrison et al. 1989) where a panicogenic agent was administered both in the FP and in the LP, vulnerability to developing a panic attack following $CO_2$ inhalation was increased in PMDD women relative to controls in both the FP and LP of the MC. However, because of the small number of PMDD patients who agreed to inhale $CO_2$ in both phases, the authors were unable to draw any conclusions regarding a phase-related difference in reactivity to panicogenic agents in PMDD women. To assess any putative phase effects, CCK-4 was administered to women during both the FP and LP.

Another objective of this study was to examine whether the behavioral effects provoked by CCK receptor activation are altered by the MC phases and their concomitant gonadal hormonal changes. Changes in CCK plasma concentrations have been detected during the MC in humans and during the estrous cycle in rats. For example, in healthy women, plasma CCK concentrations are more elevated during the LP of the menstrual cycle relative to the FP (Frick et al. 1990). In female rats, CCK binding, CCK availability, and the number of CCK-immunoreactive neurons in different central sites, vary according to the estrous cycle and following administration of estradiol (Goldman et al. 1984; Akesson et al. 1987; Micevych et al. 1988; Oro et al. 1988). For example, proestrus female rats show a greater number of cholecystokinin immunoreactive cells in the posterodorsal nucleus of the amygdala (Oro et al. 1988). Recent experiments in rats have revealed that the pharmacological manipulation of female gonadal hormones alters the effects of CCK on food intake and lordosis (Geary et al. 1994; Ulibarri and Micevych 1993; Mendelson and Gorzalka 1994; Wagner-Srdar et al. 1987). Interestingly, the CCK content (CCKi) of the amygdala, a central neuroanatomical structure involved in anxiety response, varies during the estrous cycle and is maximal during estrogen peaks of the cycle (see Micevych and Ulibarri, 1992 for review). Likewise, the panicogenic effects of CCK agonists might be expected to vary with hormonal changes. Preliminary results related to portions of this study have been published elsewhere in the form of a letter (Le Mellédo et al. 1995).

## SUBJECTS AND METHODS

### Subjects

Eighteen women with DSM-IV PMDD (mean age: 31 ± 7 years) and 21 control women (mean age: 27 + 7 years) who responded to newspaper advertisements participated in the study after providing oral and written informed consent. All subjects were physically healthy as determined by medical history, physical examination, electrocardiogram, and routine laboratory tests. Subjects were evaluated with the Structured Clinical Interview for *DSM-III-R* for nonpatients (SCID-NP). Women with PMDD were free of any current Axis I psychiatric diagnoses and a lifetime history of bipolar disorder, psychotic disorders, anxiety disorders, or somatoform disorders. We cannot report any data on the coprevalence of these disorders with PMDD, because the exclu-

sion took place before PMDD was diagnosed with prospective monitoring of premenstrual symptoms. Because of the high prevalence of depression in PMDD, women with a history of major depression were included in the study provided that their last episode remitted at least 2 years prior to the screen visit. None of the control women had a current or lifetime history of Axis I psychiatric disorders as determined by the SCID-NP. No subjects had a history of panic attacks or a first-degree relative with panic disorder. Subjects were excluded from the study if they: (1) had serious medical disorders; (2) were taking any medication; (3) smoked more than 15 cigarettes a day; (4) drank more than five cups of coffee a day; (5) were pregnant or lactating; (6) had given birth in the previous 6 months; (7) had an abortion in the previous 3 months; (8) had irregular menstrual cycles; (9) had an average menstrual cycle length greater than 35 days or less than 24 days; and (10) had used or discontinued hormonally based contraceptives in the previous 3 months.

The presence or absence of PMDD was ascertained by the prospective monitoring of at least four completed MCs using a modified Prospective Record of the Impact and the Severity of Menstrual Symptomatology (PRISM) (Reid and Robinson 1985) and 100-mm visual analogical scales (VAS). Our modification of the PRISM calendar consisted of replacing the item "restlessness" (described by many experts in the field as being not very informative) by the item "overwhelmed" (a new DSM-IV item not included in the pre-DSM-IV PRISM calendar). The range of scoring of every item was rated between 1 (not present) and 7 (very severe) on the version of the PRISM available at the time of the study. Subjects completed the PRISM calendar every day throughout the MC. Two VAS were completed during each MC, the first one was completed 7 to 10 days after the onset of menses and the second 1 to 5 days before the onset of the next menses, which was prospectively calculated according to the length of each woman's MC. Our instructions to the subjects were to complete their second VAS between 2 and 5 days before the expected date of menses. Unfortunately several shorter than expected MCs resulted in several VAS completed only 1 day prior the onset of menses. We assessed the presence of DSM-IV PMDD criteria based on the PRISM calendar (which ensured that premenstrual emotional symptoms were not limited to the day of the LP VAS completion). The menstrual cyclicity and severity of "mood symptoms" were objectively verified by comparing the ratings of the VAS ratings during the FP and the LP. Affect cyclicity was ensured by a within-cycle (FP to LP) increase of at least 50% in three menstrually related mood symptoms (tension, dysphoria, mood swings and irritability) or a 100% increase in the severity of one of these symptoms. For the increase in severity to be considered clinically significant, the severity of menstrual symptoms had to be greater than 40 mm on the VAS scale during the LP. This 40-mm cut-off score is still accepted in the new National Institutes of Mental Health (NIMH) guidelines (NIMH task force on PMDD, 1997). These requirements had to have been present for at least half of the MCs monitored in each subject. We did not use a maximal cut-off for the FP ratings, because the NIMH guideline for PMDD research in effect at the time we designed this study did not recommend an FP cut-off score. Recently, an FP cut-off score has been added to the NIMH guidelines to exclude women who present significant emotional symptoms during their FP. In our study, women with consistent severe emotional symptoms during the FP would have been excluded for current depressive episode or current anxiety disorder following the performance of the Structure Clinical Interview (SCID) that was systematically administered during the FP, 5 to 10 days after the onset of the menses.

The NIMH guidelines for premenstrual syndrome (1983) were applied retrospectively to the PRISM ratings. These guidelines require that the sum of the LP ratings during the 6 days prior to menses "should be" 30% greater than the sum of the FP ratings between day 5 and 10 after the onset of menses. All the PMDD women included in this study met NIMH criteria for premenstrual syndrome that confirmed the severity and the cyclicicity of their premenstrual symptoms.

Group assignment had not been made at the time of the placebo injection. The group assignment was performed during the third visit, following the monitoring of two complete MCs.

### Design

The study employed a placebo-controlled cross-over design. Each subject participated in three sessions and received one placebo injection and two CCK-4 injections. All subjects received placebo first, which coincided with the LP of the MC. The two CCK-4 injections corresponded to the FP (7 to 10 days after the onset of menses) and LP (1 to 5 days prior to menses). The order of menstrual phase (i.e., whether CCK-4 was administered first during the FP or the LP) was counterbalanced across subjects. The use of placebo on the first test day allowed subjects to accommodate to the experimental procedure and reduced the probability of dropouts between the two CCK-4 sessions (to 0). Subjects were blind to the number of placebo and CCK-4 injections they received and to the menstrual phase order in which placebo and CCK-4 were administered. Menstrual phase was confirmed using a urine luteinizing hormone (LH) detection kit (Clearplan Easy, CIBA Unipath, Ltd.). This test was intended to help the scheduling of the LP visit by ensuring that the LP injection was performed after the LH peak. We cannot state the study

days with respect to the day of the LH surge, because we found at several occasions discrepancies between absence of LH peak (suggesting an absence of ovulation) and high progesterone levels during the LP (suggesting that ovulation took place). The study was approved by the St. Mary's Hospital Ethics Committee.

## ASSESSMENT

### Behavioral Analyses

A DSM-III-R derived Panic Symptom Scale (PSS) (Bradwejn et al. 1991) was used to characterize behavioral responses to the CCK-4 (and placebo) challenge. The panic symptoms on this scale were the following: "feeling short of breath/and or smothering sensation," "dizziness," "unsteady feeling," "faintness," "palpitations and/or rapid heart," "trembling and/or shaking," "sweating," "choking feeling," "nausea," "abdominal distress," "feeling unreal and/or detached from your body," "numbness and/or tinglings in part of your body," "hot flashes and/or cold chills," "chest pain and/or discomfort," "anxiety, fear, and/or apprehension," "fear of dying," "fear of losing control," "fear of going crazy." As in their previous studies, to avoid interference between subjects' basal states and postinjection ratings, this research group systematically instructed subjects, in a standardized fashion, to rate these items with respect to the change from the way they were feeling before the injection. Subjects were directed to rate the severity of these 18 panic symptoms as either absent (0), mild (1), moderate (2), severe (3) or extremely severe (4). Two separate scores were obtained from this scale: (1) a sum intensity score (i.e., the sum of the intensity ratings); and (2) a score reflecting the total number of symptoms reported (i.e., number of symptoms with scores $>1$). The occurrence of panic attacks following CCK-4 administration was determined based on the DSM-IV criteria for panic attacks and based on a score of two or more on the PSS "anxiety, fear, apprehension" item. At the end of the study, subjects were asked to compare their response to the two CCK-4 injections. The duration of the panic symptoms was determined based on the subjects indication and timed by the blind rater (in this study an increase of 30 s represents an increase of approximately 1/3 of the total duration of the symptoms).

### Cardiovascular Analyses

Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded using an automatic sphingomanometer (every 20 s for the first 5 minutes following the injections).

### Biochemical Analyses

Blood sampling for measurement of: CCK, estradiol (E), progesterone (P), luteinizing hormone (LH), and follicular hormone (FSH) took place 1 h before injection and 45 min after IV installation.

***Methods for Total CCK-Like Immunoreactivity (CCK-LI) Measurements.*** CCK-4 was obtained from Peptides International, Louisville, KY.

*Antisera and Tracer Preparation*: Plasma cholecystokinin levels were analyzed by a radioimmunoassay using antisera directed against the cholecystokinin tetrapeptide (Merani et al. 1997). Antiserum against CCK-4 was prepared by conjugation to thyroglobulin by the carbodiimide method (Vaitukaitis et al. 1971). Briefly, 25 mg of thyroglobulin in 0.5 ml of distilled water ($dH_2O$) was added to 5 mg of CCK-4 dissolved in 0.5 ml of distilled water and adjusted to pH 5.5. 5 mg of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide-HCL (CDI) in 0.4 ml of $dH_2O$ was added dropwise with constant mixing and incubated overnight at 4°C. 35 mg of CDI was added and mixed at room temperature for 2 h. Excess coupling agent was removed by 24-h dialysis against 2 l of 0.001 M phosphate buffer (pH 7.4) containing 0.9% NaCl. The antigenic solution (100 mg/ml saline) was emulsified in complete Freund's adjuvant and injected intradermally to New Zealand White rabbits. Intramuscular injections of 0.5 ml Bordetella pertussis vaccine were administered as nonspecific stimulus. Booster injections of 50 mg of the antigen emulsified in incomplete Freund's adjuvant were given every 4 weeks for 8 months (Skowsky and Fisher 1972). Bolton Hunter CCK-4 was iodinated using a previously described method designed to achieve high tracer yield (Tower et al. 1977).

*Sample Extraction and Radioimmunoassay*: Plasma was extracted using Sep-Pak C18 cartridges (Waters). Cartridges were activated with 8 ml acetonitrile and washed with 8 ml $dH_2O$. Samples were applied to cartridges, washed with 5 ml $dH_2O$ and eluted with 3 ml 70% EtOH. The eluant was dried in a speed-vac (Savant) overnight. Lyophilized samples were reconstituted in assay buffer (0.1M NaCl, 1% BSA, 1% Triton X-100, 0.1% sodium azide in 0.1 m phosphate buffer, pH 7.4) and were added in duplicate 100 ml aliquots to polystyrene tubes at 4°C. This assay buffer was used for all subsequent dilutions. The standard curve consisted of 100 ml duplicates of CCK-4 in the range of 0.4 to 400 pg prepared by 1:2 serial dilutions in buffer. 100 ml or 200 ml of assay buffer was added to tubes to test for total binding or nonspecific binding (NSB) respectively. 100 ml of antiserum (diluted 1:10,000) was added to each tube except the NSB tubes and incubated at 4°C for 24 h. 100 ml of tracer (6,000 to 8,000 cpm) was then added to each tube and incubated as above. 100 ml of normal rabbit serum (1:35) and 100 ml goat antirabbit g-globulin (1:50) were added and incubated at 25°C for 2 h. Tubes

were centrifuged at 3,000 rpm at 4°C for 20 min after the addition of 1 ml of polyethylenglycol. Precipitate radioactivity was counted upon aspiration of supernatant.

*Results*: The antibody employed in the immunoassay was raised against the CCK tetrapeptide and is equipotent for the CCK tetrapeptide and octapeptide (Merani et al. 1997). Thus the CCK values reported in this manuscript represent these total CCK measurements. The range of the standard curve was determined to be 0.4 to 400 pg CCK-4/tube (0.7 to 672 fmol CCK-4 equivalents/tube). Intra-assay and interassay coefficients of variation were determined to be 8 and 9% respectively (average value characteristic for this particular radioimmunoassay at approximately 30% binding). However, for this particular study, all samples were analyzed in one assay, thus negating any interassay variation. Specific activity of iodinated BH CCK-4 was calculated as ~1,025 Ci/mmol. Binding of tracer in absence of standard (zero binding) was 15.3 = ±0.2% at a 1:10,000 antisera dilution, with half-maximal displacement (ED50) at 38.5 = ±1.4 fmol. Nonspecific binding was calculated as 1.4 = ±0.1%.

***Methods for Measurement of E, P, LH, and FSH.*** E, P, LH, and FSH were measured in the department of Clinical Biochemistry at the Hôpital Ste-Justine, Université de Montréal. Total serum E and P were measured with solid-phase double antibody immunofluorometric assays. The intra- and interassay coefficients of variation were, 3.0 and 5.0% at 0.9 nmol/l and 2.4 and 2.9% at 10.9 nmol/l respectively. LH and FSH were measured with solid-phase two-site fluorometric assays in which two mouse monoclonal antibodies are directed against two separate epitopes (Wallac Canada). The intra- and interassay coefficients of variation for LH were, respectively, 1.6 and 2.3% at 7 I.U./L and, 0.8 and 3.0% for a FSH concentration of 6.7 I.U./L. In the aforementioned assays AutoDelfia technology was used to measure the fluorescence generated by the tracer Europium (Wallac Canada, Kirkland, Quebec).

## PROCEDURE

This study consisted of five visits (V1, V2, V3, V4, V5). V1 involved psychological and physical screening; an explanation of how to complete the PRISM calendar and associated VAS; and the planning of V2. V2 included the LP placebo injection. Randomization of subjects to the phase order of the CCK-4 injections took place during V3, when at least two complete MCs had elapsed since study enrollment. A list for randomization was generated in blocks of six. The procedures of V2 (placebo injection), V4 (first CCK-4 injection), and V5 (second CCK-4 injection) were identical. Shortly after arrival, subjects sat on a reclining chair and an IV catheter was installed into their antecubital vein through which a NaCl 0.9% solution was slowly dripped. Bolus placebo and CCK-4 were administered through the catheter at least 1.5 h postcatherization.

## MATERIAL

### Cholecystokinin-Tetrapeptide

CCK-4 was purchased from Peninsula (California), and a sterile solution was prepared by GIS Médicament (Nantes, France) according to previous protocols (Bradwejn et al. 1991). The use of CCK-4 was approved by the Health Protection Branch of Health and Welfare Canada. The placebo consisted of an identical volume (1.75 ml) of 0.9% NaCl solution.

## DATA ANALYSIS

To compare the effects of CCK-4 and placebo on the behavioral, physiological, and biochemical outcome variables, we used the data from the LP phase only, because placebo was only given during that phase, and we performed a three-way analysis of the variance (ANOVA) model with repeated measurements on one factor (Winer 1971). The repeated factor was treatment (placebo vs. CCK-4), and the two between subject factors were diagnosis (control vs. PMDD) and sequence (CCK-4 injection during phase LP at visit 4 vs. at visit 5). All the interactions were included in the model. For the binary outcome "panic," we used the generalized estimating equations (GEE) approach (Zeger and Liang 1986) with the same model as for the continuous variables. To compare the effects of the FP versus the LP on CCK-4 injection response, we used a linear model for crossover design (Jones and Kenward 1989). In this model, the main effects of interest were: (1) diagnosis (control vs. PMDD); (2) sequence in which the subjects received the CCK-4 injections (i.e., FP–LP vs. LP–FP); (3) phase (FP vs. LP); (3) and visit (V4 vs. V5). We also included in the model all the double interactions with the diagnosis factor. The same model was applied for the analysis of the binary outcome "panic" using the GEE method. Finally, for the categorical variable "phase preference," a chi-square test was performed to test the hypothesis of no phase preference for both groups separately. We considered as statistically significant $p$-values less than 5%. All statistical analyses were conducted using SAS statistical software version 6.12 for Windows 95.

## RESULTS

Eight women received a placebo injection (V2) and did not receive CCK-4 injections (V4). Because of their early

drop out, these 8 women were not adequately monitored, which would have allowed us to identify clearly their assignment group. We describe below their most probable diagnosis as well as the reasons why they did not receive the first CCK-4 injection. Five women who contacted the unit and were screened as healthy volunteers were unable to return for the following visits; one woman who contacted the unit as a PMDD woman and had a first PRISM calendar compatible with the diagnosis of PMDD became depressed; one PMDD woman was turned-off of the study because we could not install the IV catheter to proceed with the first CCK-4 injection; and one likely healthy volunteer showed poor compliance in completing the PRISM calendars.

Randomization of the CCK-4 injection phase order resulted in nine of the 18 PMDD women receiving their first CCK-4 injection during the FP and nine receiving their first CCK-4 injection during the LP (9 FP–LP, 9 LP–FP). Among the 21 controls, 12 received their first CCK-4 injection during the FP and nine during the LP (12 FP–LP, 9 LP–FP). Despite the unpleasant experience following the first CCK-4 injection (V4), all women agreed to return for the next and last visit (V5).

The means and standard deviations of the behavioral and physiological outcome variables for each diagnosis, phase, and sequence are reported in Table 1. The panic rates are shown in Figure 1.

## Comparison between the Effects of CCK-4 and Placebo During the LP

Table 2 presents the results of the three-way analysis of variance with repeated measurements on one factor. For all outcome variables considered, there is a highly statistically significant increase in the response to CCK-4 as compared to placebo. All other effects and interactions are not statistically significant except for the symptom "anxiety, fear, apprehension," where there is a diagnosis main effect and an interaction diagnosis by treatment ($p$-values = .02 and .01, respectively) indicating a significantly greater response to CCK-4 in PMDD women for that specific symptom. Figure 1 shows that no women experienced a panic attack during the placebo injection contrasting with 67% in the PMDD group and 38% in the control group during the LP CCK-4 injection. This difference between panic rates in the two groups was only marginally significant ($p = .08$).

## Comparison between the 2 CCK-4 Injections

Table 3 illustrates the results of the linear modeling analysis for the cross-over trial.

***Differences between PMDD and Controls.*** There are statistically significant higher levels of: "anxiety, fear, apprehension"; duration of panic symptoms; and panic rates in PMDD women ($p = .011$, .049, and .002, respectively) (see Table 4).

***Menstrual Cycle Phase Effects on the Response to the CCK-4 Effects in PMDD Women and Controls.*** Although only marginally significant, the results of this analysis suggest, after adjusting for the possible confounding visit and sequence effects, a greater response during the LP for the "anxiety, fear, apprehension" and the PSS SI scores (estimate of the phase effect LP–FP were 0.37, $p = .077$ and 2.17, $p = .106$ respectively). Similarly, the $p$-value for diagnosis by phase of 0.075 for ΔHR reveals a trend toward a higher increase in heart rate in PMDD women during the LP. Analysis of the

**Table 1.** PSS SI Score, Duration, Number of Panic Symptoms and Physiological Changes Following Placebo and CCK-4 Injections

| | Descriptives Statistics: Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| | Control FP $n = 21$ | Control LP $n = 21$ | | PMDD FP $n = 18$ | PMDD LP $n = 18$ | |
| Effects of CCK-4 Injections | CCK-4 | Placebo | CCK-4 | CCK-4 | Placebo | CCK-4 |
| Anxiety/fear/ apprehension | 1.0 ± 1.3 | 0.2 ± 0.5 | 1.1 ± 1.3 | 1.7 ± 1.1 | 0.2 ± 0.4 | 2.3 ± 1.5 |
| PSS SI score | 18.4 ± 11.1 | 2 ± 2.6 | 18.7 ± 11.1 | 21.6 ± 11.1 | 3.6 ± 4.8 | 25.4 ± 14.9 |
| Duration of panic | 119 ± 43 | NA | 108 ± 39 | 139 ± 53 | NA | 139 ± 51 |
| Number of panic symptoms | 8.9 ± 3.5 | 1.3 ± 1.5 | 8.6 ± 3.8 | 9.2 ± 3.7 | 2.6 ± 2.5 | 10.3 ± 4.4 |
| Δ HR (bpm) | 30 ± 11 | 6 ± 6 | 29 ± 13 | 29 ± 14 | 6 ± 10 | 35 ± 14 |
| Δ SBP (mm Hg) | 17 ± 11 | 7 ± 4 | 16 ± 11 | 16 ± 9 | 6 ± 6 | 16 ± 9 |
| Δ DBP (mm Hg) | 12 ± 9 | 4 ± 7 | 8 ± 9 | 11 ± 7 | 6 ± 10 | 11 ± 7 |

Analysis of the diagnosis, phase and diagnosis × phase effects on CCK-4 injections, NA = not applicable, PSS SI score = sum intensity of panic symptoms, Δ HR = maximal increase in heart rate, Δ SBP = maximal increase in systolic blood pressure, Δ DBP = maximal increase in diastolic blood pressure.

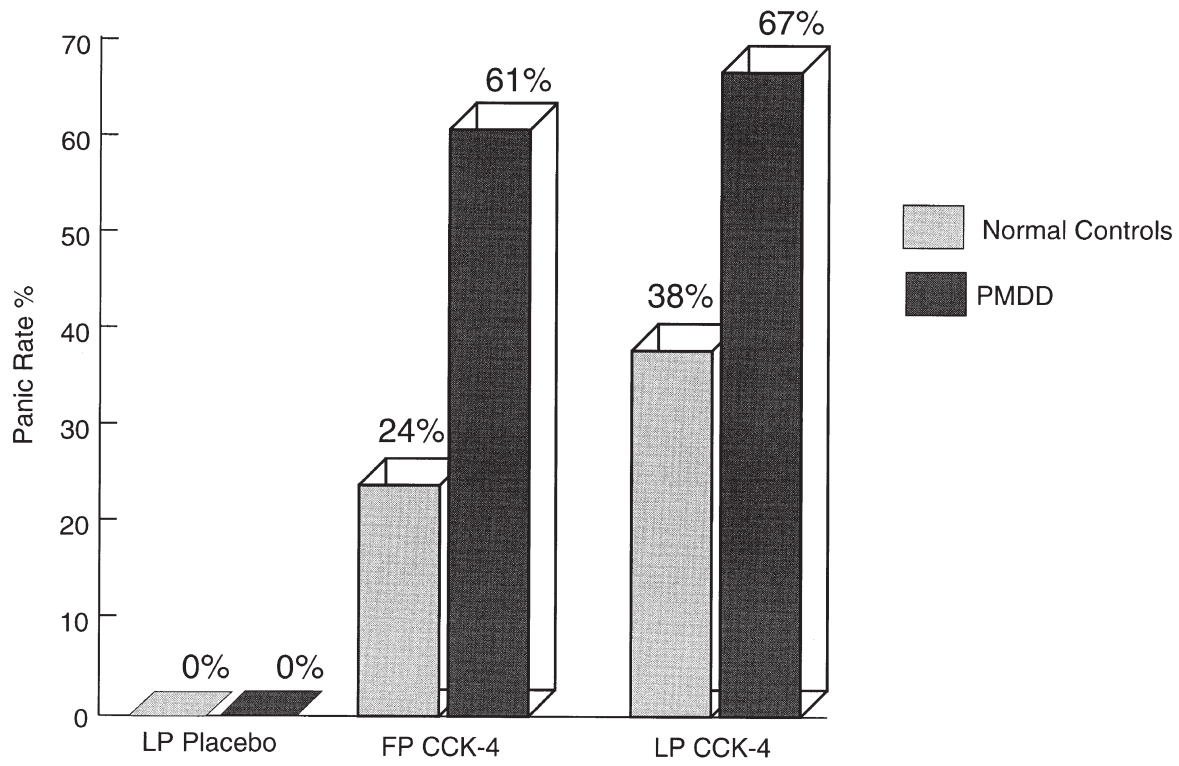


**Figure 1.** Panic rate response to placebo and CCK-4 injections; FP: follicular phase; LP: luteal phase.

subjective impression (phase preference) for the effects of CCK-4 injections revealed that more PMDD women found the LP CCK-4 injection more intense ($\chi^2(1) = 6.33$, $p = .04$). 61% (11) of PMDD women found the LP CCK-4 injection to be more intense; 22% (4) found the FP injection to be more intense; and 17% (3) found no difference between the two injections. Among controls, 48% (10) found the LP CCK-4 injection to be more intense, 38% (8) found the FP injection to be worse, and 14% (3) found no difference at all. The phase preference for control subjects did not reach significance ($\chi^2 = 3.71$, $p = .16$).

**Table 2.** Comparison Between Placebo and CCK-4 Injection During the Luteal Phase (LP)

| | ANOVA | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Anxiety** | **PSS SI Score** | **Duration** | **Number of Symptoms** | **HR** | **SBP** | **DBP** |
| Diagnosis | F = 5.96 | F = 3.02 | F = 1.87 | F = 2.09 | F = 0.88 | F = 0.10 | F = 1.33 |
| | $p = .020$ | $p = .091$ | $p = .101$ | $p = .098$ | $p = .355$ | $p = .757$ | $p = .256$ |
| Treatment (Pl vs. CCK-4) | F = 38.89 | F = 105.57 | F = 115.96 | F = 174.99 | F = 144.25 | F = 33.42 | F = 8.36 |
| | $p < .001$ | $p < .001$ | $p < .001$ | $p < .001$ | $p < .001$ | $p < .001$ | $p < .007$ |
| Diagnosis × treatment | F = 7.45 | F = 1.57 | F = 2.23 | F = 0.09 | F = 2.04 | F = 0.01 | F = 0.07 |
| | $p = .010$ | $p = .218$ | $p = .145$ | $p = .772$ | $p = .162$ | $p = .915$ | $p = .791$ |
| Sequence (LP V4 vs. LP V5) | F < 0.01 | F = 2.47 | F = 0.85 | F = 2.67 | F = 2.89 | F = 1.29 | F = 4.15 |
| | $p = .974$ | $p = .125$ | $p = .364$ | $p = .111$ | $p = .099$ | $p = .264$ | $p = .049$ |
| Diagnosis × sequence | F = 1.33 | F = 1.32 | F = 5.16 | F = 1.72 | F = 0.78 | F = 0.09 | F = 0.58 |
| | $p = .257$ | $p = .259$ | $p = .029$ | $p = .198$ | $p = .384$ | $p = .772$ | $p = .450$ |
| Treatment × sequence | F = 0.04 | F = 2.87 | F = 0.87 | F = 3.07 | F = 0.47 | F = 2.58 | F = <0.001 |
| | $p = .842$ | $p = .099$ | $p = .358$ | $p = .089$ | $p = .497$ | $p = .118$ | $p = .984$ |
| Diagnosis × treatment × sequence | F = 0.24 | F = 0.03 | F = 1.80 | F = 0.01 | F = 0.18 | F = 1.14 | F < 0.01 |
| | $p = .628$ | $p = .859$ | $p = .188$ | $p = .923$ | $p = .670$ | $p = .294$ | $p = .991$ |

PSS SI score: panic sum intensity, V: visit, Sequence: LP V4 = the CCK-4 injection during the LP is the first CCK-4 injection, LP V5 = the CCK-4 injection during the LP is the second CCK-4 injection. Δ HR = maximal increase in heart rate, Δ SBP = maximal increase in systolic blood pressure, Δ DBP = maximal increase in diastolic blood pressure. All tests performed in Table 2 have 35 df.

**Table 3.** Comparison Between the Two CCK-4 Injections

| | ANOVA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Anxiety** | **PSS SI Score** | **Duration** | **Number of Symptoms** | **HR** | **SBP** | **DBP** | **Basal Plasma CCK.Li** |
| Diagnosis | F = 7.32 | F = 1.69 | F = 4.16 | F = 0.65 | F = 0.28 | F = 0.09 | F = 0.10 | F = 0.03 |
| | $p = .011$ | $p = .202$ | $p = .049$ | $p = .424$ | $p = .560$ | $p = .767$ | $p = .756$ | $p = .875$ |
| Phase (FP vs. LP) | F = 3.33 | F = 2.75 | F = 0.42 | F = 1.08 | F = 1.09 | F = 0.23 | F = 2.40 | F = 0.38 |
| | $p = .077$ | $p = .106$ | $p = .520$ | $p = .305$ | $p = .304$ | $p = .638$ | $p = .131$ | $p = .541$ |
| Visit (V4 vs. V5) | F = 0.86 | F = 5.41 | F = 3.46 | F = 2.16 | F = 0.52 | F = 2.26 | F = 3.00 | F = 4.11 |
| | $p = .359$ | $p = .026$ | $p = .071$ | $p = .150$ | $p = .474$ | $p = .141$ | $p = .092$ | $p = .05$ |
| Sequence (FP/LP vs. LP/FP) | F = 0.15 | F = 1.12 | F = 0.11 | F = 2.35 | F = 1.63 | F = 0.66 | F = 0.73 | F < 0.001 |
| | $p = .697$ | $p = .297$ | $p = .737$ | $p = .135$ | $p = .210$ | $p = .442$ | $p = .398$ | $p = .981$ |
| Diagnosis × phase | F = 1.45 | F = 1.71 | F = 0.37 | F = 2.57 | F = 3.37 | F = 0.05 | F = 3.00 | F = 0.05 |
| | $p = .236$ | $p = .199$ | $p = .546$ | $p = .118$ | $p = .075$ | $p = .828$ | $p = .092$ | $p = .820$ |
| Diagnosis × visit | F = 0.01 | F = 1.32 | F = 1.02 | F = 0.02 | F = 0.82 | F = 0.74 | F = 0.69 | F < 0.01 |
| | $p = .918$ | $p = .258$ | $p = .320$ | $p = .883$ | $p = .371$ | $p = .397$ | $p = .413$ | $p = .944$ |
| Diagnosis × sequence | F = 1.26 | F = 0.18 | F = 0.20 | F = 0.94 | F = 0.01 | F = 0.09 | F = 0.05 | F = 0.02 |
| | $p = .268$ | $p = .676$ | $p = .661$ | $p = .339$ | $p = .941$ | $p = .770$ | $p = .820$ | $p = .903$ |

FP: follicular phase, LP: luteal phase, V4: visit 4, V5: visit 5, Sequence FP/LP: the first CCK-4 injection took place during the FP, Sequence LP/FP: the first CCK-4 injection took place during the LP. PSS SI score: panic sum intensity, Δ HR = maximal increase in heart rate, Δ SBP = maximal increase in systolic blood pressure, Δ DBP = maximal increase in diastolic blood pressure. Δ HR = maximal increase in heart rate, Δ SBP = maximal increase in systolic blood pressure, Δ DBP = maximal increase in diastolic blood pressure. All tests performed in Table 2 have 35 df.

***Baseline CCK-Li Measurements.*** We found significantly lower levels of CCK-Li at V5 as compared to V4 (0.050) but no other main effect.

***Hormones.*** No differences in E, P, FSH, LH plasma levels were found between diagnoses, and there were no correlations between concentrations of these hormones and the response to CCK-4.

## DISCUSSION

Our results demonstrate that women with PMDD exhibit a greater anxiety and panic response to CCK-4 as compared to control women during both the FP and the LP. They also suggest that an enhanced general behavioral sensitivity to CCK-4 exists in PMDD women. Indeed, in our PMDD women, the duration of panic symptoms was greater (with statistical significance), the PSS SI and number of panic symptoms were greater (showing both marginal significance and nonsignificance in the two analyses performed). Our behavioral results are in accordance with those of previous studies, which suggested that women with severe premenstrual symptoms have an enhanced vulnerability to other panicogenic agents, sodium lactate (Fachinetti et al. 1992; Sandberg et al. 1993), and $CO_2$ (Harrison et al. 1989).

The absolute value of the outcome variable means favors a greater reactivity to CCK-4 during the LP than the FP in PMDD but a strict statistical significance was found only for the subjective impression of worst injection. The phase effect was only marginally significant for other variables such as anxiety and PSS SI score. This apparent (although inconclusive) greater reactivity to CCK-4 during the LP of PMDD lends clinical relevance to our findings, because the mid-FP is, by definition, symptom free. The enhanced CCK-4 sensitivity of PMDD women during the nonsymptomatic mid-FP is

**Table 4.** Basal Plasma CCK-Li for Every Diagnosis (PMDD and Controls), in Every Phase (FP and LP) for Visit 4 and 5 (V4, V5), for Every Sequence (Order of CCK-4 Injections FP/LP vs. LP/FP)

| | **Controls** | | | | **PMDD** | | | |
|---|---|---|---|---|---|---|---|---|
| | **FP** | | **LP** | | **FP** | | **LP** | |
| | **V4 CCK-4 (FP/LP) $n = 12$** | **V5 CCK-4 (LP/FP) $n = 9$** | **V4 CCK-4 (LP/FP) $n = 9$** | **V5 CCK-4 (FP/LP) $n = 12$** | **V4 CCK-4 (FP/LP) $n = 9$** | **V5 CCK-4 (LP/FP) $n = 9$** | **V4 CCK-4 (LP/FP) $n = 9$** | **V5 CCK-4 (FP/LP) $n = 9$** |
| CCK Plasma Levels pg/ml | 10.60 ± 9.8.00 | 7.99 ± 7.43 | 10.27 ± 10.03 | 7.29 ± 3.52 | 10.74 ± 8.00 | 7.87 ± 4.63 | 9.34 ± 5.96 | 7.01 ± 3.67 |

FP = follicular phase, LP = luteal phase, PMDD = women with premenstral dysphoric disorder.

not a surprising find. It is consistent with the results obtained in other biological challenge studies of PMDD women (Bancroft et al. 1991; Harrison et al. 1989) and leads us to the state-versus-trait issue (Bancroft 1993), which needs to be addressed in future investigations. The differences in behavioral and physiological responses between placebo and CCK-4 injections in healthy volunteers are consistent with those already discussed in many studies (Bradwejn et al. 1991; De Montigny 1989; Jerabek et al. 1995).

CCK-LI plasma levels, independent of diagnosis or phase, were higher before the first CCK-4 injection than before the second CCK-4 injection. Based on increased blood-borne CCK described in sportsmen before a competitive marathon run, as compared with control conditions (Philipp et al. 1992), the difference that we observed could be explained by the fact that prior to a perceived stressful event, subjects were in a different affective or arousal state. This state might have been different if women had known what to expect from an earlier experience with CCK-4 (i.e., before the second CCK-4 injection) compared to when they faced an unknown event (i.e., before the first CCK-4 injection). The novelty of the situation might have induced an increased arousal and/or apprehension that translated into greater CCK-LI plasma levels. The lack of phase effect that we observed on the CCK-LI plasma levels is at odds with Frick and co-workers' (1990) findings of increased CCK-LI plasma levels during the LP versus the FP. In our study, this phase influence might have been overshadowed by the stronger "affective state effect" of potentially anticipatory anxiety, related to the visit effect.

Our baseline CCK-Li levels contribute little to the discussion of the still controversial mechanism of action involved in the anxiogenic activity of peripherally administered CCK-4. Indeed, it has never been demonstrated that CCK-4 crosses the blood brain barrier. The lack of diagnosis $\times$ phase effect for CCK-Li levels suggests, however that CCK-Li plasma level changes are not associated with the usual increase in baseline anxiety observed in the LP of PMDD women (Stein et al. 1989).

In summary, our preliminary results show that the anxious and panic response to CCK-4 is greater in PMDD women than in controls. Our findings also suggest that an enhanced sensitivity to CCK-4 exists in PMDD women during the LP. This hypothesis however requires further testing with a greater sample size, because few variables demonstrated strict statistical significance. Our preliminary results are consistent with a specific biological reactivity of PMDD women to CCK-4. These results call for additional studies on the cause of this hypersensitivity to CCK in PMDD women in which the physiology of CCK and the interaction between the CCK system and other neurotransmitter systems can be investigated in detail. The study of the potential therapeutic benefits of CCK-B antagonists and CCK-A agonists (because of their putative CCK-B antagonist activity) in PMDD seems worthwhile. Our results also suggest that, when conducting panic challenge studies and clinical trials, one should consider controlling for women with PMDD and menstrual phase. Furthermore, our findings suggest that the anxiety component of PMDD is as relevant as the depressive component.

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