Premenstrual Dysphoric Disorder in Relation to Neuroactive Steroids and Alcohol

Sigrid Nyberg

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To My Family

What ever you think is important is worth reaching for
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PAPERS I - V
Introduction: Premenstrual Dysphoric Disorder (PMDD) is a condition that affects about 2-6% of women of reproductive age. The relation to ovarian steroids is apparent as symptoms are absent during anovulatory cycles. Neuroactive steroids like allopregnanolone have effect in the brain and on brain function and have been proposed to play an important role for the symptomatology of premenstrual symptoms and in the interaction between the GABA_A receptor and alcohol. High doses of alcohol elevate allopregnanolone levels both in rats and humans. Allopregnanolone is a positive modulator of the GABA_A receptor with sedative, anxiolytic and anticonvulsant effect in both human and animals.

Aims: The aim was to investigate if a low dose (100μg) of GnRH agonist (buserelin) is effective for the treatment of PMDD and if allopregnanolone serum levels during treatment are associated to symptom severity. Furthermore, the studies aimed at investigating the effect of a low dose of alcohol upon saccadic eye movements in women with PMDD, and control subjects in different phases of the menstrual cycle, and to evaluate if there was a difference in response to alcohol between men and healthy women. We also wanted to see if this low dose of alcohol could have an effect on serum allopregnanolone levels in women with PMDD and control subjects in the follicular and luteal phases of the menstrual cycle.

Methods: The effect of low dose (100μg) of GnRH agonist (buserelin) on premenstrual symptoms was evaluated in a randomized, placebo-controlled, double-blinded cross-over trial. 27 PMDD patients were randomized to either GnRH agonist intranasally once a day or placebo for two months before the crossover. The main outcome measure was the daily symptom ratings for mood and physical symptoms made by the patients. In a subgroup of 12 women, grouped as buserelin responders and placebo responders, luteal phase serum progesterone, allopregnanolone, and pregnanolone was measured together with daily ratings for mood and physical symptoms. Alcohol responsiveness was measured in PMDD patients, female control subjects and men by comparing the effect of a low dose (0.2g/kg) of intravenous alcohol or placebo infusion upon saccadic eye movements. Blood samples for measurement of allopregnanolone and cortisol were taken throughout the alcohol/placebo challenges.

Results: Low dose GnRH agonist was effective as treatment of premenstrual irritability and depression. Anovulatory cycles were confirmed in 56% of the subjects, particularly in older women. Buserelin as well as placebo responders displayed decreased allopregnanolone and progesterone levels in parallel with symptom improvement. PMDD patients displayed blunted saccadic eye movement response to alcohol infusion, especially in the luteal phase. Control subjects did not change their response to alcohol between cycle phases. We found no difference in saccadic eye movement sensitivity to alcohol between males and females. Allopregnanolone levels significantly decreased in the luteal phase following the alcohol infusion.

Conclusions: Low dose GnRH agonist is effective in treatment of premenstrual depression and irritability but is likely to induce anovulation with increasing age. Independent of whether buserelin or placebo treatment was given decreased levels of allopregnanolone appear to be related to symptom improvement. Women with PMDD have altered saccadic eye movement sensitivity in response to alcohol, particularly in the luteal phase. The low dose of alcohol did not induce any difference in saccade measurements between males and females. Low dose of alcohol does not result in increased peripheral levels of allopregnanolone.

Key words: Premenstrual Dysphoric Disorder, GnRH-agonist, progesterone, allopregnanolone, alcohol, saccadic eye velocity.
# ABBREVIATIONS

<table>
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<th>Abbreviation</th>
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<tr>
<td>ACOG</td>
<td>American college of obstetricians and gynecologists</td>
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<td>ACTH</td>
<td>adrenal corticotropic hormone</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>BAC</td>
<td>blood alcohol concentration</td>
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<td>BrAC</td>
<td>breath alcohol concentration</td>
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<td>CCK</td>
<td>cholecystokinin</td>
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<td>CD</td>
<td>cyclicity diagnoser</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<td>CRH</td>
<td>corticotrophin-releasing hormone</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CSGAAS5</td>
<td>Cardiff saccade generation and analysis system</td>
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<tr>
<td>DSM-IV</td>
<td>diagnostic and statistical manual of mental disorders, fourth edition</td>
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<td>EOG</td>
<td>electro-oculography</td>
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<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<tr>
<td>GABA</td>
<td>gamma aminobutyric acid</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; receptor</td>
<td>gamma aminobutyric acid type A receptor</td>
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<td>GnRH</td>
<td>gonadotropin-releasing hormone</td>
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<tr>
<td>HPA-axis</td>
<td>hypothalamus-pituitary-adrenal axis</td>
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<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>HT</td>
<td>hormonal therapy</td>
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<td>LEDs</td>
<td>light-emitting diodes</td>
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<td>LH</td>
<td>luteinizing hormone</td>
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<td>LLPDD</td>
<td>late luteal phase dysphoric disorder</td>
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<tr>
<td>NAD&lt;sup&gt;+&lt;/sup&gt;</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
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<td>NADH</td>
<td>reduced nicotinamide adenine dinucleotide</td>
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<td>PD</td>
<td>panic disorder</td>
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<td>PMDD</td>
<td>premenstrual dysphoric disorder</td>
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<td>PMS</td>
<td>premenstrual syndrome</td>
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<tr>
<td>RIA</td>
<td>radio immunoassay</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>SEM</td>
<td>standard error of mean</td>
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<td>SEV</td>
<td>saccadic eye velocity</td>
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<tr>
<td>SSRI</td>
<td>selective serotonin re-uptake inhibitor</td>
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<td>VAS</td>
<td>visual analogue scale</td>
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<tr>
<td>3α5α-THDOC</td>
<td>3α5α-tetra-hydro-desoxycorticosterone</td>
</tr>
<tr>
<td>5α-DHP</td>
<td>5α-dihydroprogesterone</td>
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptophan, serotonin</td>
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ORIGINAL PAPERS


II Nyberg S, Bäckström T, Zingmark E, Sundström-Poromaa I. Allopregnanolone decrease with symptom improvement during placebo and GnRH agonist treatment in women with premenstrual dysphoric disorder. Submitted manuscript.


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INTRODUCTION

Premenstrual Dysphoric Disorder

Definition and Diagnosis

Premenstrual Dysphoric Disorder (PMDD) is characterized by physical and psychological symptoms appearing in the luteal phase of the menstrual cycle. The symptoms often start at ovulation or shortly after and continue, mostly with an increase in severity, until bleeding starts. The severity of the symptoms reaches the highest levels during the last five premenstrual days and on the first days of bleeding and disappears within a few days after the bleeding starts (Bäckström et al., 2003). However, symptoms between cycles can vary within women in both onset and duration (Pearlstein et al., 2005). In the context of this thesis, it is important to distinguish between prospectively defined premenstrual dysphoric disorder and self-reported premenstrual syndrome (PMS). When asked prospectively, more than 90% of women report cyclicity in at least one symptom (mental or physical) during the menstrual cycle (Sveindottir and Bäckström, 2000). Two-thirds of women in reproductive age retrospectively report mental symptoms and feelings of body swelling during the premenstrual phase. Of these, 10.8% wanted to consult a physician because of their premenstrual symptoms (Andersch et al., 1986). However, only 2-6% of the women meet the prospective criteria for PMDD (Sveindottir and Bäckström, 2000) as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, DSM-IV (American Psychiatric Association, 1994). In 2000, the DSM-IV was revised (DSM-IV-TR) with no changes in the diagnostic criteria for PMDD.

Several twin studies suggest that there might be a hereditary component in premenstrual complaints (Dalton et al., 1987; van den Akker et al., 1987; Condon, 1993; Kendler et al., 1998) although environmental risk factors can also contribute to premenstrual symptoms. Most of these studies retrospectively assess symptoms, an approach that should be considered when analyzing the data.

Premenstrual symptoms appear to deteriorate as women age. In the Zurich 10-year prospective cohort study (Merikangas et al., 1993), a positive association between increase in premenstrual symptoms and increase in age (from 21 to 30 years of age) was noted. In a population-based study of women aged 18 to 44 years (Deuster et al., 1999), symptoms were most evident in women 25 to 34 years old (10.4%) compared to women 18 to 24 years old (8.7%) and women 35
to 44 years old (4.5%). Similar findings are reported in a study of women in their late twenties through mid-thirties, (Freeman et al., 1995a). As women approach menopause, the symptom severity seems to decline (Ramcharan et al., 1992).

The most common symptoms reported by women with PMDD are irritability, depressed mood, anxiety, mood lability, and tension (Hurt et al., 1992; Steiner et al., 1997; Eriksson, 1999). These symptoms should be verified using prospective ratings because retrospective ratings are less valid. Many studies have demonstrated the discrepancy between retrospective reports of premenstrual complaints and reports based on daily prospective evaluation. Retrospective assessment favors recall of premenstrual symptoms, whereas symptoms at other times across the menstrual cycle are forgotten. Studies have shown that between 14 and 50% of women who complain about premenstrual symptoms do not show a true relation between the menstrual cycle and cyclicity of symptoms (Hurt et al., 1992). As well as confirming the presence of symptoms in the luteal phase, it is necessary to confirm the absence of symptoms in the follicular phase to distinguish the syndrome from other current mood disorders such as major depression, generalized anxiety disorder, and panic disorder. Patients with an underlying affective disorder can experience a premenstrual aggravation of their symptoms (Endicott, 1993). In addition to the presence of a number of typical symptoms in the luteal phase, the DSM-IV criteria also state that symptoms must interfere with usual activities (school, work performance, or interpersonal relationships).

Confusing Terminology

During the past two decades, the terminology referring to premenstrual syndrome has changed several times. Originally, the term “premenstrual syndrome” was used although the diagnostic criteria varied substantially between researchers. Because there was a consensus for the need for prospective symptom ratings in the early 1980s, the first diagnostic criteria were established for what then was called the “late luteal phase dysphoric disorder” (LLPDD), a description found in the appendix of Diagnostic and Statistical Manual of Mental Disorder-III-R (DSM-III-R) (American Psychiatric Association 1987). Importantly, some researchers adhered to the term PMS while using the LLPDD criteria. Later the term LLPDD was changed to premenstrual dysphoric disorder (PMDD, in the beginning sometimes also abbreviated as PDD) in a following edition (DSM-IV) (American Psychiatric Association 1994). As some women did not fulfill the criteria for PMDD but still required treatment for their condition, various descriptions for syndromes
with less number of symptoms have been suggested such as “premenstrual dysphoria” (PMD) and premenstrual syndrome (as defined on the next page).

In this thesis, most references refer to studies where criteria for PMDD have been used, although some researchers might have called it PMS, LLPDD, or PDD. If diagnosis was based on DSM-III-R or DSM-IV criteria I have used the term PMDD in this thesis, independent of which terminology that originally was used by the authors. When the term PMS is used in this thesis, these studies either have used the new PMS criteria (from 2000) or I have not been able to ascertain that DSM-criteria was used. If diagnosis is based on retrospective reporting, I note this to indicate that these findings are less valid.

Research Criteria for Premenstrual Dysphoric Disorder

A. In most menstrual cycles during the past year, five (or more) of the following symptoms were present most of the time during the last week of the luteal phase, began to remit within a few days after the onset of the follicular phase, and were absent in the week post menses, with at least one of the symptoms being either (1), (2), (3), or (4):

(1) markedly depressed mood, feelings of hopelessness, or self-deprecating thoughts;

(2) marked anxiety, tension, feeling of being “keyed up”, or “on edge”;  

(3) marked affective lability (e.g., feeling suddenly sad or tearful or increased sensitivity to rejection);  

(4) marked and persistent anger or irritability or increased interpersonal conflicts;  

(5) decreased interest in usual activities (e.g., work, school, friends, hobbies);  

(6) subjective sense of difficulty in concentrating;  

(7) feeling lethargic, easy fatigability, or marked lack of energy;  

(8) marked change in appetite, overeating, or specific food cravings;  

(9) hypersomnia or insomnia;
(10) a subjective sense of being overwhelmed or out of control;

(11) other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, a sensation of “bloating”, and weight gain;

B. significant interference with work or school or with usual social activities and relationships with others (e.g. avoidance of social activities, decreased productivity and efficiency at work or school);

C. feelings that are not merely an exacerbation of the symptoms of another disorder, such as major depressive disorder, panic disorder, dysthymic disorder, or a personality disorder; and

D. criteria (A), (B), and (C) confirmed by prospective daily ratings during at least two consecutive symptomatic cycles.

Diagnostic Criteria for Premenstrual Syndrome

Many women do not fulfill the DSM-IV criteria for PMDD although their symptoms are severe enough to influence their daily life and to seek medical treatment. For this reason, a definition of premenstrual syndrome was recently proposed by the American College of Obstetricians and Gynecologists (ACOG 2000). Although not employed in this thesis, the criteria are given for comparison.

Premenstrual syndrome can be diagnosed if the patient reports at least one of the following affective and somatic symptoms during the 5 days before menses in each of the three prior menstrual cycles.

Affective
- Depression
- Angry outbursts
- Irritability
- Anxiety
- Confusion
- Social withdrawal

Somatic
- Breast tenderness
- Abdominal bloating
- Headache
- Swelling of extremities
These symptoms are relieved within 4 days of the onset of menses without recurrence until at least cycle day 13. The symptoms are present in the absence of any pharmacologic therapy, hormone ingestion, or drug or alcohol use. The symptoms occur reproducibly during two cycles of prospective recording. The patient suffers from identifiable dysfunction in social or economic performance. Few studies have used the new definition of premenstrual syndrome and only a few such studies are included in this thesis.

Influence on Daily Life

The main symptoms for PMDD are mental and affect daily living and well-being in a significant way. The impairment of the quality of life as well as the disability adjusted life years lost in PMDD is in the same magnitude as in depressive disorders (Pearlstein et al., 2000; Halbreich et al., 2003). The burden of PMDD has been estimated to 3.8 years of disability over the reproductive years for each woman with PMDD (Halbreich et al., 2003). The premenstrual impairment may be more severe at home, affecting the relationship with family members more than social and work functioning (Kuczmicz et al., 1992; Frank et al., 1993; Campbell et al., 1997; Brown et al., 1998; Pearlstein et al., 2000; Robinson and Swindle, 2000). Chawla and co-workers (2002) found that women with PMDD and PMS had greater impaired work productivity without affecting the time at work, in the luteal phase. In a community-based study on 1045 women using telephone interviews regarding premenstrual symptoms and impact on functioning and treatment-seeking behavior, Hylan and colleagues (1999) reported that functional impairment was more significant at home than in social, school, or occupational situations. These findings point out that these women might increase their efforts to cope with their symptoms at work and in school and do not allow themselves to let it interfere until they are at home with their family.

Women with PMDD often report that they experience a subjective feeling of altered cognitive functioning during the luteal phase with deteriorated concentration, attention, and memory (Man et al., 1999). Most studies on cognitive functioning in women with PMDD have not supported these findings. No differences were found in verbal learning and memory between women with prospectively diagnosed PMDD and controls; however, women with PMDD demonstrated slower psychomotor control in the late luteal phase (Resnick et al., 1998). Working memory was impaired in the luteal phase of the menstrual cycle with no significant differences between PMDD and control subjects (Man et al., 1999). PMDD women had significant difficulty in learning new material, but this problem was not phase-dependent and mood did not account for any of the differences in cognitive functioning (Keenan et al.,
Other studies have found no difference in cognition when comparing
women with PMDD and controls (Rapkin et al., 1989; Morgan et al., 1996;
Morgan and Rapkin, 2002). These studies suggest that although women with
PMDD report subjective feelings of diminished cognition premenstrually,
there is no objective evidence that this is the case. Morgan and Rapkin also
suggest that these complaints could be due to altered perceptions and
sociocultural expectations.

Data suggests that premenstrual mood symptoms increase the prevalence of
suicidal thoughts and suicidal attempts during the premenstrual phase
(Chaturvedi et al., 1995; Wittchen et al., 2002; Baca-Garcia et al., 2004). One
limitation to these studies is the retrospective diagnosis of the premenstrual
symptoms, which influences the interpretability of these findings.

Pathophysiology of PMDD – Hormonal Aspects

The pathophysiology of PMDD is unclear, but the presence of ovarian
hormones is considered crucial in the syndrome because during anovulatory
cycles, when no corpus luteum is formed in the ovary, the symptoms do not
appear (Hammarbäck et al., 1991). Progesterone has been suggested as the
major symptom-provoking factor because of its temporal relationship with the
luteal phase and its adverse mood effects during sequential hormone therapy
in postmenopausal women (Björn et al., 2000; 2002; 2003; Andréen et al., 2003;
2005; Wihlbäck et al., 2001; 2005). When postmenopausal women are treated
with estrogen-only, no negative mood effects are seen; however, when
estradiol therapy is combined with progesterone or progestogens, negative
mood symptoms appear (Hammarbäck et al., 1985; Björn et al., 2000; Andréen
et al., 2003; 2005; 2006).

In addition, estradiol appears to provoke premenstrual symptoms. Schmidt
and co-workers evaluated women with PMDD and controls to measure mood
response with respect to hormone levels. A GnRH analog, administered to
obtain ovarian suppression, improved symptom ratings for the PMDD
patients. When either estradiol or progesterone was added to the GnRH
treatment, the negative symptoms returned in the PMDD patients, but not in
the controls. This was interpreted as a general abnormal response to normal
hormonal fluctuations in PMDD patients (Schmidt et al., 1998). In PMDD
patients high levels of luteal phase estradiol seems to be related to more severe
symptoms (Hammarbäck et al., 1989a; Wang et al., 1996; Seippel et al., 1998).
Furthermore, a higher dose of estrogen in combination with progestogen
results in more negative effects on mood than a lower dose in postmenopausal
women (Björn et al., 2003).
Most studies have found no difference in peripheral levels of progesterone and estradiol in the luteal phase between PMDD patients and controls (Bäckström et al., 1983; Rubinow et al., 1988; Girdler et al., 1993). Similarly, no differences in follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, or cortisol have been reported (Bäckström et al., 1983; Rubinow et al., 1988; Girdler et al., 1993); however, in some studies on women with PMDD, higher levels of testosterone are evident compared to controls (Eriksson et al., 1992; 1994).

Although women with PMDD appear not to have higher stress levels (Beck et al., 1990), they are less tolerant to stress than other women (Girdler et al., 1998; Deuster et al., 1999). Hypothalamus, the pituitary gland, and the adrenal glands (HPA-axis) regulate the body’s response to stress. The adrenal hormone cortisol, which is a glucocorticoid hormone, plays a key role in stress reduction by its effect on several body systems. Both over-activity and under-activity of the HPA-axis function are associated with depressive mood states and aggressive behavior (Yehuda et al., 1993; Stansbury and Gunnar, 1994). Reduced HPA-axis activation has been documented among women with PMDD in several studies (Roy-Byrne et al., 1986; Redei and Freeman, 1993) and women who felt more depressed premenstrually had lower salivary cortisol levels compared to postmenstrual days (Odber et al., 1998).

Pathophysiology of PMDD – Psychiatric Aspects

There are similarities between PMDD and affective disorders like major depression, generalized anxiety disorder, and panic disorder (PD) (Yonkers et al., 1997a,b). Compared to controls, the lifetime prevalence of depressive disorder and postpartum depression is increased in women with PMDD (Endicott and Halbreich, 1988; Pearlstein et al., 1990; Hurt et al., 1992). The prevalence of life-time history of major depressive disorder in women with PMDD is reported to be between 30-80% (Halbreich and Endicott, 1985; Harrison et al., 1989). PMDD in itself has also been suggested to be a risk factor for future major depressive disorder (Graze et al., 1990; Hartlage et al., 2001).

Apart from the similarities between PMDD and major depression, PMDD patients also share several common biological vulnerabilities with PD patients. Exposure to different anxiety-producing agents like lactate, CO₂, and cholecystokinin (CCK) induce panic attacks in both women with PD and women with PMDD (Facchinetti et al., 1992; Le Mellédo et al., 1999; Gorman et al., 2001). A possible influence of gonadal hormones has been suggested since
panic symptoms seem to increase in the post-partum period (Northcott and Stein, 1994) and in the late premenstrual period (Yonkers et al., 1997c) with decreasing levels of progesterone. During pregnancy, a time of high levels of both estrogen and progesterone, improvement in PD has been reported (Cohen et al., 1994), although this result was not confirmed in a following study (Cohen et al., 1996). A greater respiratory variability among subjects with PD has been seen at baseline (Gorman et al., 1988; Abelson et al., 2001) and during carbon dioxide (CO₂) challenge (Bystritsky et al., 2000). Similar changes in respiratory variability has been seen both at baseline (Martinez et al., 2001) and after CO₂ inhalation among women with PMDD compared to control subjects (Bystritsky et al., 2000; Martinez et al., 2001). In addition, both PMDD and PD patients display a reduced sensitivity to a benzodiazepine challenge (Roy-Byrne et al., 1990; Sundström et al., 1997a,b; Le Mellédo et al., 2001), and both disorders respond to SSRIs (Ballenger et al., 1988; Harrison et al., 1990; Modigh et al., 1993; Freeman et al., 1995b; Steiner et al., 1995).

Another area of research that has gained increasing interest is the prevalence of sexual abuse and domestic violence in women with PMDD. Golding and Taylor (1996), using two survey data sets with 948 and 619 women, found an association between retrospective reports of premenstrual distress and repeated assaults by the same offender. Golding and co-workers (2000), in a prospective interview of 42 women with confirmed PMDD, found that 40 out of 42 women reported at least one attempted or completed sexual assault. Paddison and colleagues (1990) interviewed 174 women seeking treatment for prospectively defined premenstrual symptoms about their history of sexual abuse; 40% of these women had been sexually abused. These findings suggest that there is a strong association between earlier traumatic events and premenstrual symptoms. Finally, Girdler and colleagues reported that women with PMDD with a history of sexual abuse are distinct from other women with PMDD and from women without PMDD in terms of variability and mean hormone concentrations of hypothalamus-pituitary-thyroid axis variables (2004).
PMDD and Neurosteroids

Several neurotransmitter systems – the serotonergic (Parry, 2001), the noradrenergic (Halbreich et al., 1993), and the GABAergic system (Sundström et al., 1997b; Epperson et al., 2002; Wihlbäck et al., 2006) – have been implicated in the pathogenesis of PMDD. The main focus of this thesis is the GABA system.

Sex steroid hormones play fundamental roles in the development and adult function of the central nervous system (CNS). Generally, steroid hormones, such as cortisol and progesterone, exert their effects over a relatively long time by acting as transcription factors that regulate protein expression. CNS acts both as a source and a target of sex steroids and of their metabolites. Recent findings indicate that there is a complex interplay of genomic and non-genomic signaling mechanisms of steroid hormones. In the 1990s, numerous studies have indicated that steroids or neuroactive metabolites of steroids can alter the neuronal excitability and synaptic function through direct membrane mechanisms, such as ligand-gated ion channels and neurotransmitter transporters (Wong et al., 1996).

![Figure 1](image.png)

**Figure 1.** The theoretical model of the GABA\(_{\alpha}\)-receptor complex.

Endogenous neuroactive steroids are A-ring reduced metabolites of the steroid hormones progesterone, deoxycorticosterone, and testosterone. Progesterone serves as the precursor for the neuroactive steroids allopregnanolone (3α-hydroxy-5α-pregn-20-one) and pregnanolone (3α-hydroxy-5β-pregn-20-
Neurosteroids are synthesized from cholesterol in the brain or from steroid hormone precursors imported from peripheral sources (Stoffel-Wagner, 2001; Ottander et al., 2005).

In 1986, it was shown that allopregnanolone and $3\alpha,5\alpha$-tetra-hydrodesoxycorticosterone ($3\alpha,5\alpha$-THDOC, a metabolite of deoxycorticosterone) could modulate neuronal excitability via their interaction with GABA$_A$ receptors (Majewska et al., 1986). The GABA-transmitter system is the major inhibitory system in the mammalian CNS. When GABA binds to the receptor, the influx of chloride ions renders the post-synaptic cell less prone to excitation. The GABA$_A$ receptor consists of five subunits, forming a ligand-gated chloride channel, (Figure 1) (Luddens and Wisden, 1991). The five subunits are comprised of varying combinations of $\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, $\pi$, and $\rho$ (Hevers and Luddens, 1998) with the most functional receptors containing $\alpha / \beta / \gamma$, or $\alpha / \beta / \delta$ subunits (McKernan and Whiting, 1996; Davies, 1997). Recently, $\delta$ subunit-containing GABA$_A$ receptors have been suggested to be selectively involved in neurosteroid-mediated extrasynaptic inhibition (Stell et al., 2003).

![Figure 2](image-url) The pathway of allopregnanolone synthesis in the brain, with progesterone as precursor.

The GABA$_A$ receptors are the site of action of various pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anesthetics, anticonvulsants, and alcohol (Mehta and Ticku, 1999). Importantly, for this thesis, alcohol has been shown to have a specific binding site at the GABA$_A$ receptor (Mihic, 1999).
Although neurosteroids, such as allopregnanolone, can be synthesized de novo in the central nervous system (Stoffel-Wagner, 2001), in fertile women it is conceivable that the major contributor to endogenous levels of allopregnanolone is the corpus luteum, where progesterone is produced during the luteal phase of the menstrual cycle (Bäckström et al., 1986; Ottander et al., 2005), Figure 2. Both in serum and brain tissue, the levels of allopregnanolone are following the levels of progesterone over the menstrual cycle (Wang et al., 1996; Bixo et al., 1997; Genazzani et al., 1998). In fertile women, plasma levels of allopregnanolone are approximately 0.3 - 2 nmol/L in the follicular phase and 1 - 4 nmol/L in the luteal phase. In the third trimester of a pregnancy, these levels increase up to more than 100 nmol/L (Bicikova et al., 1995; Luisi et al., 2000).

Most substances active at the GABA\(_A\) receptor will induce tolerance when used long-term; this is also true for allopregnanolone (Zhu et al., 2004; Turkmen et al., 2006). Tolerance to GABA-active neurosteroids might contribute to the symptoms in women with mood disorders and explain the decreased sensitivity to both pregnanolone (Sundström et al., 1998b) and benzodiazepines previously reported in PMDD patients (Sundström et al., 1997b). Animal studies have shown that after chronic administration of allopregnanolone a down regulation of the GABA\(_A\) receptor appears (Yu et al., 1996) and a decreased sensitivity at the GABA\(_A\) receptor to benzodiazepines and neurosteroids becomes evident (Smith et al., 1998). A withdrawal effect has also been suggested to induce mood disorders when high levels of allopregnanolone during pregnancy and in the luteal phase of the menstrual cycle decline.

Several studies have indicated that allopregnanolone influences mood, behavior, stress response, and cognitive functions. Major depression has been associated with low allopregnanolone serum concentrations (Romeo et al., 1998; Ströhle et al., 2000; Nappi et al., 2001). Romeo and colleagues also found that the low levels of allopregnanolone in depressed patients were normalized after treatment with fluoxetine. Similar results were seen in cerebrospinal fluid (CSF) of patients with unipolar major depression where treatment with fluoxetine resulted in improved symptoms together with increasing CSF levels of allopregnanolone (Uzunova et al., 1998). A significant decrease in postpartum allopregnanolone levels was seen in women with postpartum “blues,” but there was no correlation between the levels of progesterone and allopregnanolone, whereas a positive correlation was seen in women who did not experience the “blues” (Nappi et al., 2001), suggesting that allopregnanolone might attribute to the symptoms by different conversion of 3α-reduced neuroactive steroids in depression (Ströhle et al., 1999; 2000).
In patients with mixed anxiety-depressive disorder (Bicicova et al., 2000) or generalized anxiety disorder (Semeniuk et al., 2001), no difference in allopregnanolone levels was seen compared to controls; however, in women with panic disorder elevated allopregnanolone levels was reported in both the follicular and luteal phase of the menstrual cycle (Brambilla et al., 2003).

Based on the discrepancy in findings regarding peripheral allopregnanolone concentrations, PMDD appears not to be due to a deficiency or excess of allopregnanolone. In some studies, lower luteal phase allopregnanolone levels were seen in PMDD patients (Rapkin et al., 1997; Bicikova et al., 1998; Monteleone et al., 2000), but in other studies higher levels (Girdler et al., 2001) or similar levels (Schmidt et al., 1994; Wang et al., 1996; Sundström et al., 1998b) were detected. In addition, serum and plasma levels of pregnanolone (a stereoisomer to allopregnanolone), pregnenolone or pregnenolone sulfate are the same for PMDD patients and control subjects (Schmidt et al., 1994; Wang et al., 1996; Sundström et al., 1998b). Improvement of premenstrual symptoms, whether induced by SSRI treatment or placebo, has been associated with lower levels of allopregnanolone (Freeman et al., 2002). Again, this finding is in contrast with other studies which have reported that higher luteal phase concentrations of allopregnanolone are associated with improved symptoms (Wang et al., 1996; Girdler et al., 2001).

Allopregnanolone and other GABA<sub>A</sub> receptor agonists – such as, benzodiazepines, barbiturates, and alcohol – exert a bimodal effect on mood and behavior in certain individuals. With high concentrations, these agonists enhance the effect of GABA, leading to sedative, anxiolytic, antiepileptic, and anesthetic effect in both animals and humans (Carl et al., 1990; Sundström and Bäckström, 1998b). Low concentrations, however, induce negative mood, loss of impulse control, and aggression (Cherek et al., 1992; Dougherty et al., 1996; Ben-Porath and Taylor, 2002; Miczek et al., 2003). A hypothetical theory for this paradox in the effect of different doses is a mechanism called disinhibition, which means that the suppression of the inhibitory effect at the GABA<sub>A</sub> receptor complex may increase excitability. In a group of women tested with three doses of alcohol (0.25, 0.50, and 1.0 g/kg of body weight), the highest dose significantly increased aggressive response compared with placebo. However, in a subset of these women, aggressive response was mostly increased after the lowest alcohol dose (Dougherty et al., 1996).

PMDD diagnosis seems to be important for the allopregnanolone response to stress. Compared to controls, women with PMDD did not show the expected rise in allopregnanolone levels following mental stress nor did they show a positive correlation between allopregnanolone and progesterone concentrations (Klatzkin et al., 2005). Whereas 83% of healthy controls
responded with the expected stress-induced increase in allopregnanolone following a speech and a paced auditory serial addition stress test (PASAT), only 42% of the PMDD patients did respond (Girdler et al., 2001). An increase in plasma allopregnanolone concentration is normally seen after tests stimulating ovarian and adrenal function but a reduction in allopregnanolone response was found in women with PMS when tested in the luteal phase with a GnRH- and ACTH-test (Lombardi et al., 2004). This result was interpreted as an altered production of ovarian neuroactive steroids during the luteal phase.

Using saccadic eye velocity and subjective scores of sedation as independent measures, patients with PMDD have been reported to have a decreased responsiveness to pregnanolone and benzodiazepines (Sundström et al., 1997a,b; 1998a). Moreover, neurosteroid and benzodiazepine insensitivity were also related to symptom severity within the PMDD group (Sundström et al., 1997a,b; 1998a). High-severity PMDD patients were even less responsive to GABA-modulatory neurosteroids and benzodiazepines than low-severity patients (Sundström et al., 1997a,b; 1998a), and neurosteroid insensitivity was restored after treatment with SSRI (Sundström and Bäckström, 1998c).

Because of the reduced sensitivity to GABA-ergic compounds, response to alcohol (also acting via the GABA<sub>A</sub> receptor) is of interest to evaluate in PMDD patients.

**Treatment**

There are two main pharmacological treatments for PMDD: 1) the induction of anovulation or 2) modulation of serotonergic transmission. Both options have benefits and adverse effects. There are several ways to induce anovulation, such as GnRH agonist treatment, (Hammarbäck et al., 1988), high doses of estrogen (Watson et al., 1989), danazol (Halbreich et al., 1991), medroxyprogesterone in high doses (Kaunitz, 1998), and surgical oophorectomy (Casper and Hearn, 1990). In this work, I have only focused on GnRH agonists and SSRIs.

**SSRIs**

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter used by many neurons throughout the brain. Serotonin may influence aggression, impulse control, anxiety, sexual behavior, pain, sleep, and appetite (Spigset, 1997). Serotonin alters the function in different neurons by altering the rate of signaling and also modulates the release of other neurotransmitters. Several
studies have indicated that serotonin reuptake inhibitors (SSRIs) are effective in treatment of PMDD, (Sundblad et al 1992; Steiner et al., 1995; Eriksson et al., 1995; Yonkers et al., 1997c; Pearlstein et al., 1997; Wikander et al., 1998; Freeman et al., 1999; Dimmock et al., 2000; Cohen et al., 2002) and these compounds are now considered as a drug of choice.

SSRIs reduce symptoms like irritation and depressed mood (Sundblad et al., 1992; Eriksson et al., 1995; Pearlstein et al., 1997; Steiner et al., 1999; Wyatt et al., 2002), but also seem to have an effect on physical symptoms like breast tenderness and bloating (Eriksson et al., 1995; Freeman et al., 1999). Typically, treatment with SSRIs results in improvement in psychosocial functioning, quality of life, and work capacity (Pearlstein et al., 2000; Steiner et al., 2003). Intriguingly, the mechanism of action seems to be different from the mechanism by which the SSRIs alleviate symptoms in major depression. When used for treatment of depression, the lag phase (time from onset of treatment to treatment effect) for SSRIs are usually 4-8 weeks (Wikander et al., 1998; Freeman et al., 1999). When used for PMDD, only a few days are needed until symptom improvement is noted (Steiner et al., 1995). The treatment is effective also when given intermittently, during the luteal phase (Sundblad et al., 1993; Halbreich and Smoller, 1997; Young et al., 1998; Wikander et al., 1998; Jermain et al., 1999; Cohen et al., 2002; Halbreich et al., 2002; Freeman et al., 2004; 2005), and data suggests that intermittent treatment might be even more effective than continuous treatment (Steiner et al., 1997; Wikander et al., 1998). Intermittent dosing supposedly minimizes the adverse effects (Steiner et al., 1997; Sundblad et al., 1997) and is also more cost beneficial.

In animal studies, both fluoxetine and paroxetine seem to change the brain level of allopregnanolone and 5α-dihydroprogesterone (5α-DHP) without affecting the levels of other neurosteroids (Guidotti et al., 1996; Uzunov et al., 1996). This effect of SSRIs on allopregnanolone biosynthesis has been suggested to be mediated by changes in enzymatic activity of 3α-hydroxysteroid oxidoreductase (the enzyme that synthesizes allopregnanolone from 5α-DHP), (Uzunov et al., 1996). Possibly, this modulation of allopregnanolone brain levels could contribute to the rapid onset effect of SSRIs in PMDD patients.

Further evidence indicating altered serotonin function in PMDD patients include studies where decreased blood serotonin levels (Rapkin et al., 1987), decreased platelet serotonin content (Ashby et al., 1988), and increased binding to the serotonin transporter (Bixo et al., 2001) have been found in women with PMDD. A significant correlation between changes in perceived irritability and depressed mood in the luteal phase compared to follicular phase and changes in brain serotonin precursor (L-tryptophan) trapping were
recently reported in women with premenstrual dysphoria (Eriksson et al., 2006).

Although the effect of SSRIs is excellent, approximately 50% of the women do not continue the treatment after six months due to the side effects of treatment (Sundström-Poromaa et al., 2000). The most commonly reported side effects of SSRIs are sexual dysfunction such as reduced libido and anorgasmia. These side effects are also the most common cause why patients discontinue their prescription (Pearlstein and Stone, 1994; Sundström-Poromaa et al., 2000). Other common side effects are nausea and sweating. Alternatives to treatment with SSRIs are often requested by the afflicted women.

GnRH-agonists

The hypothalamus produces gonadotropin-releasing hormone (GnRH), a hormone that stimulates the pituitary gland to release and synthesize luteinizing hormone (LH), and follicle stimulating hormone (FSH). GnRH agonists are synthetic analogues to GnRH, and buserelin is 16 to 40 times more potent than endogenous GnRH. GnRH agonists induce a down-regulation of the pituitary GnRH receptors and high doses cause hypoestrogenism.

GnRH agonists can effectively treat PMDD (Muse et al., 1984; Hammarbäck and Bäckström, 1988; Mortola, 1993). However, although the induced anovulation ameliorates symptoms, it also results in postmenopausal concentrations of plasma estrogen and progesterone. Undesirable side effects of menopausal symptoms – such as hot flushes, vaginal dryness, insomnia and depression - often make the treatment intolerable for many women. Long-term treatment also increases the risk of osteoporosis, why it is necessary to use estrogen and progestogen as additional therapy. While PMDD symptoms can return during add-back hormone therapy, the symptom relief is still more pronounced compared to placebo (Mortola et al., 1991; Studd and Leather, 1996; Schmidt et al., 1998).

GnRH agonist treatment appears to be specific for premenstrual dysphoric disorder. No effect was seen with GnRH agonist (leuprolide) in patients with premenstrual exacerbation of major depression, a finding that suggests a different underlying mechanism of PMDD and major depression (Freeman et al., 1993; 1997).

In gynecologic practice, GnRH agonists are used to treat estrogen dependent conditions, such as endometriosis and uterine fibroids. The recommended
daily dose for treatment of endometriosis with nasal GnRH agonist (Suprefact 100μg/dose) is 900 μg.

To overcome the disadvantages of GnRH agonist therapy, alone or in combination with add-back hormone therapy (HT), low-dose GnRH could provide an alternative approach. Low dose GnRH agonist therapy would, hypothetically, reduce corpus luteum function without affecting the ovulatory mechanisms. As a consequence of a possible reduction in corpus luteum function, estradiol and progesterone concentrations would decline without becoming completely suppressed, thus reducing part of the symptom-provoking agents in the luteal phase.

PMDD and Alcohol Consumption

Heavy alcohol consumption has physical, psychological, social, and economic consequences.
Several studies have identified risk factors for alcohol abuse in women. Childhood physical and sexual abuse seems to be more common among women with substance-use in general (Simpson and Miller, 2002), and women seeking alcohol treatment also are more likely to report earlier abuse (Rice et al., 2001; Bentdtsen et al., 2002). Also, affective disorders are often co-morbid with alcoholism (Peindl et al., 1998).

PMDD is yet another potential risk factor for alcohol abuse or modulation of drinking patterns. Women with prospectively confirmed PMDD have reported increased alcohol consumption during the luteal phase compared to control subjects (Christensen et al., 1989; Mello et al., 1990). Also, compared to control women attending a gynecologic clinic, women with PMDD are more often alcohol abusers, with prevalence rates of 12% and 21%, respectively (Halliday et al., 1986), and PMS-clinic attendee’s report more alcohol abuse than a community sample (Stout et al., 1986). Furthermore, 102 women retrospectively diagnosed with PMS reported greater alcohol consumption compared to controls, but the most frequent consumption was during the postmenstrual phase (Caan et al., 1993). Chuong and co-workers (1995) found that 39.5% of women with prospectively confirmed PMDD reported regular alcohol use, whereas only 14.8% of controls did. The combination of a paternal history of alcohol abuse and PMDD seems to add to the risk for abuse (McLeod et al., 1994). In survey studies, alcoholic women report more severe premenstrual symptoms than non-alcoholic control women (Shelley and Anderson, 1986; Price et al., 1987).
Whereas PMDD patients appear to use alcohol differently across the menstrual cycle, several studies have not found any relationship between menstrual cycle phase and alcohol consumption among women with no complaint of premenstrual mood symptoms (Sutker et al., 1983; Harvey et al., 1985; Griffin et al., 1987; Mello et al., 1990; Caan et al., 1993; Tobin et al., 1994).

**Acute Alcohol Effects in the CNS**

Because it is difficult to determine alcohol content in alcoholic drinks and because there is no standard method that defines how much alcohol is consumed, alcohol effects in the CNS are sometimes difficult to compare between studies. For example, although the term “drink” has often been used, there is no exact definition of how much alcohol one drink contains. Different countries have different opinions of how much alcohol (in grams) a drink constitutes. For instance, in Australia and Germany, a standard drink is defined as 10 grams of alcohol and in the US a standard drink contains 14 grams of alcohol (Kerr et al., 2005). In a comprehensive review of the field, Eckardt defined one standard drink as 13.5 grams of absolute alcohol (Eckardt et al., 1998). In addition, there is also a considerable variation in definition of alcohol intake. Light drinking has been defined as consumption of between 0 to 33 grams of absolute alcohol per day and moderate drinking as an intake of between 2.7 up to 82 grams per day. Ideally, to compare studies on alcohol consumption and reactions to alcohol, the amount of alcohol used should be expressed as grams of alcohol per kilogram of lean body mass.

Furthermore, researchers measure alcohol content in different ways (e.g., mmol/L, mg/dl, mg%, or ‰), making comparisons even more difficult. To make comparisons with other studies consistent, we have standardized alcohol concentrations as mmol/L in this thesis. For comparison, 4.34 mmol/L of alcohol is equal to 0.2‰ and 10.8 mmol/L corresponds to 0.5‰.

**Table 1.** Alcohol content in different beverages

<table>
<thead>
<tr>
<th>Alcohol beverages</th>
<th>Gram of alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 40 % v/v, 10 cl</td>
<td>40 gram</td>
</tr>
<tr>
<td>Wine 10 % v/v, 10 cl</td>
<td>10 gram</td>
</tr>
<tr>
<td>Beer 4.5 % w/v, 50 cl</td>
<td>22.5 gram</td>
</tr>
<tr>
<td>Beer 2.8 % w/v, 50 cl</td>
<td>14 gram</td>
</tr>
<tr>
<td>Beer 1.8 % w/v, 50 cl</td>
<td>9 gram</td>
</tr>
</tbody>
</table>
Sensitivity to Alcohol

Alcohol binds to a specific site at the GABA\textsubscript{A} receptor (Mihic, 1999) and recombinant GABA\textsubscript{A} receptors containing $\alpha_4$ and $\delta$ subunits are stimulated by very low alcohol concentrations (Sundström-Poromaa et al., 2002). In addition to the GABA system, alcohol also interacts with a variety of transmitter systems including glutamatergic (Lovingier et al., 1989), serotonergic, dopaminergic, cholinergic, and the opioid system (Eckhardt et al., 1998).

Alcohol impairs many cognitive and physical functions: memory, divided attention, reaction time, information processing, and psychomotor performance (coordination and body sway) (Jones and Jones, 1976a,b; Mills and Bisgrove, 1983; Niaura et al., 1987; Lex et al., 1988).

This thesis focuses on low doses of alcohol rather than high, intoxicating doses. Few studies examine the effects of low doses of alcohol with blood concentrations < 10 mmol/L. A subjective feeling of intoxication appears at BAC of 2 to 7 mmol/L (Starmer, 1989; Nixon, 1995). BACs of approximately 2 mmol/L can produce physiological effects in the central nervous system (Dildy-Mayfield and Harris, 1996).

Alcohol has a biphasic effect (stimulant and sedative), depending on the phase of the blood alcohol curve and the individual (Pohorecky, 1977; Holdstock and de Wit, 1998). Alcohol is incoordinating, anxiolytic, anticonvulsant, and sedative; in high doses, it is hypnotic, similar to many effects of positive modulators of GABA\textsubscript{A} receptors (Grobin et al., 1998).

Different responses to certain effects of alcohol influence alcohol use patterns and the possibility of developing alcohol-related problems. Doses that produce activation and reduction of anxiety in some humans can have aversive, sedative, or no effect in others. Individuals who experience greater stimulant-like effects from an acute dose of alcohol report greater drug liking and greater preference for alcohol compared to placebo and compared to those who experienced more sedative-like effects (de Wit et al., 1997; Duka et al., 1998). Expectancies of the alcohol effect can influence the subjective effect of alcohol (Brown et al., 1980). A reduced response to alcohol has been seen in individuals with a family history of alcoholism (Schuckit et al., 1984; 1985; 2000; Pollock, 1992). A reduced response to alcohol among males who did not have an alcoholic father increased the risk of later developing alcohol abuse (Schuckit, 1994).
Tolerance to Alcohol

Acute tolerance is defined as a reduced effect of alcohol on the descending phase as compared to the ascending phase of the BAC curve when comparisons are made at equal alcohol concentrations. This is also referred to as the Mellanby effect (Moskowitz et al., 1979) or the acute recovery effect (Vogel-Sprott, 1979). Acute alcohol tolerance can be influenced by a number of factors, such as prior drug administration and the dose administered (Hiltunen and Järbe, 1990; 1992). When the dose and/or the rate of alcohol consumption are higher than the subject’s previous experience, acute tolerance appears (Hiltunen, 1997). Even after a single low dose of alcohol, tolerance can be demonstrated to a subsequent dose of alcohol, an effect that has been considered to be a learning effect depending on adaptive changes in the brain (Goldberg et al., 1943; Tabakoff and Kiianmaa, 1982).

The function of the GABA<sub>A</sub> receptor is reduced during chronic use of alcohol or to other GABAergic substances that enhance the effect of this transmitter system (Klein and Harris, 1996; Cagetti et al., 2003). After chronic alcohol exposure, there is a down-regulation of the receptor with a reduction of binding sites and an impaired function of the GABA<sub>A</sub>-complex.

Alcohol and Women

Women become more impaired than men after consuming equivalent amounts of alcohol even when doses are adjusted for body weight (Van Thiel and Gavaler, 1988; Holman et al., 1996; Ammon et al., 1996; Hommer et al., 2001). After drinking equal amounts of alcohol, women seem to reach higher blood alcohol levels than men (Dubowski, 1976; Jones and Jones, 1976a; Goist and Sutker, 1985), although not all studies have been able to replicate these findings (Sutker et al., 1983; Marshall et al., 1983; Baraona et al., 2001). When comparing oral intake of alcohol with intravenous administration, no difference in BAC between men and women was seen after intravenous administration (Arthur et al., 1984; Goist and Sutker, 1985; Frezza et al., 1990; Baraona et al., 2001).

The reason for gender-related differences in alcohol response has been attributed to the fact that women have more body fat and less body water than men at equal body weight (Arthur et al., 1984; Goist and Sutker, 1985; Taylor et al., 1996). The first-pass metabolism (FPM) also seems to be smaller in women than men because of a lower level of the gastric alcohol-metabolizing enzyme, alcoholdehydrogenase (ADH) (Frezza et al., 1990; Baraona et al., 2001), a more rapid oxidation of alcohol in the liver, and a delayed gastric
emptying after consuming the same type of meal (Knight et al., 1997). Women also seem to have a faster elimination rate of alcohol (Dubowski, 1976; Sutker et al., 1983; Mishra et al., 1989; Kwo et al., 1998). However, Jones and Jones do not support this conclusion (1976b).

Numerous studies have investigated gender-related cognitive impairment in response to alcohol consumption, with divergent results. With high BAC, women may be more susceptible than men to alcohol’s effect on divided attention (Mills and Bisgrove, 1983) and short-term memory (Niaura et al., 1987; Jones and Jones, 1977), but body sway (Burns and Moskowitz, 1978; Mills and Bisgrove, 1983) and coordination (Wait et al., 1982) seem to be unaffected. Flight simulator tests are very demanding on short-term memory, ability to understand information, and divided attention. When tested in a flight simulator, both men and women were significantly impaired in flight performance during acute intoxication; also, after the alcohol effect had disappeared (eight hours after alcohol administration) there were no gender differences in overall impairment (Taylor et al., 1996). No difference in SEV response to alcohol ingestion, aiming for a breath peak alcohol concentration of 80mg/dl (~17mmol/L), was noted between men and women (Blekher et al., 2002).

Preferably, studies examining alcohol effects in men and women should employ standardized mg/kg doses of alcohol and use an intravenous route for administration, to overcome as many as possible of these problems.

Alcohol and the Menstrual Cycle

A number of studies have investigated the possible interaction between the menstrual cycle and the pharmacokinetic and physiological response to alcohol. The majority of these studies, however, have serious flaws in their design. Lammers et al. (1995) reviewed eleven studies made between 1975 and 1993. This review only included studies with a within-subject design, a significant difference in variation in sex steroid activity between the time points of measurements, and verified ovulation. Only three were defined as valid, King (1984) and Sutker and colleagues (1987 a and b). These three studies did not yield any evidence of a difference in BAC or absorption rate of alcohol across the menstrual cycle. A faster elimination rate in the midluteal phase (days 20 - 25) compared to the early follicular (days 2 - 7) and ovulatory phases (around day 14) was seen (Sutker et al., 1987a, b). Other studies, not included in the above review, have yielded different results. Higher BAC was seen in the premenstrual phase in some studies (Jones and Jones, 1976a,b; Blume, 1986; Johnson, 1991), whereas other studies found
higher BAC during both the ovulation and premenstrual phase (Coupe, 1991; Littrell, 1991). The majority of the studies reported no difference in BAC across the menstrual cycle (Linnoila et al., 1980; Marshall et al., 1983; Hay et al., 1984; Brick et al., 1986; Cole-Harding and Wilson, 1987; Niaura et al., 1987; Sutker et al., 1987a; Freitag and Adesso, 1993). Likewise, Mumenthaler and colleagues reported no significant difference in pharmacokinetic parameters of alcohol throughout the menstrual cycle (1999).

The majority of studies on alcohol-related cognitive, behavioral, and sedative effects have not demonstrated any significant influences of menstrual cycle phase in healthy women (Brick et al., 1986; Niaura et al., 1987; Mumenthaler et al., 2001a,b). Although alcohol consistently induced changes in measurements of drug effect, sedation, stimulant-like effect, drug-induced euphoria, dysphoria/somatic effect, and mood, no variation across the menstrual cycle was seen (Holdstock and de Wit, 2000).

Given the previously reported decreased sensitivity to GABAergic compounds and an increased sensitivity to hormonal changes during the menstrual cycle, alcohol response is of particular interest to study in women with premenstrual dysphoric disorder.

**Alcohol and Steroid Hormones**

Even moderate alcohol consumption can have negative consequences for female reproduction. Women with a higher intake of alcohol more often suffer from dysmenorrhea, amenorrhea, and irregular menstrual periods (Wilsnack et al., 1984; Becker et al., 1989; Bahamondes et al., 1994). Acute alcohol consumption results in a rapid increase in estradiol levels in premenopausal women (Sarkola et al., 1999; 2000; Mendelson et al., 1987; 1988; 1989) and in postmenopausal women who are on estrogen replacement therapy (Ginsburg et al., 1996). Consequently, even moderate consumption of alcohol can increase the risk of developing breast cancer (Willett et al., 1987; Bowlin et al., 1997; Smith-Warner et al., 1998; Hulka and Moorman, 2001).

Acute alcohol intake also leads to an increase in plasma testosterone levels (Eriksson et al., 1994; Sarkola et al., 2000; Frias et al., 2002) and a decrease in plasma androstenedione levels in premenopausal women (Eriksson et al., 1994; Sarkola et al., 2000). These changes can be observed within one hour after intake of one to six standard drinks (one drink defined as 12 g of ethanol) and are independent of dose (Sarkola et al., 2000).
A possible explanation for the increase in estradiol and testosterone is the body’s priority for alcohol oxidation leading to a decrease in the conversion rate of estradiol to estrone, testosterone to androstenedione and progesterone to 20α-dihydroprogesterone. These reversible conversions are catalyzed by 17β-hydroxysteroid dehydrogenase, an enzyme that shares the same cofactor, nicotinamide adenine dinucleotide (NAD\(^+\)), as alcohol dehydrogenase. Alcohol dehydrogenase catalyzes the oxidation of ethanol to acetaldehyde.

Another explanation is that an increase in aromatization of testosterone to estradiol can contribute to the effect (Gavaler et al., 1993).

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} & \xrightarrow{\text{alcohol dehydrogenase}} \text{CH}_3 - \text{C} - \text{H} \\
\text{NAD}^+ & \rightarrow \text{NADH}
\end{align*}
\]

**Figure 3.** Alcohol oxidation.

**Alcohol and Neurosteroids**

From animal studies there is evidence that neuroactive steroids can play a role in a number of behavioral effects of alcohol (Morrow et al., 2001), such as anticonvulsant effects (VanDoren et al., 2000), sedation (Khisti et al., 2003), impairment of spatial memory (Morrow et al., 2001), and alcohol reinforcement (Janak et al., 1998). Allopregnanolone is rewarding in rodents and might also contribute to the rewarding and anxiolytic effects of alcohol (Finn et al., 1997).

Systemic and acute administration of high doses of alcohol (1 - 3.5 g/kg) dramatically elevates peripheral and cerebral cortical levels of allopregnanolone in both male and female rats (Morrow et al., 1998; Barbaccia et al., 1999; VanDoren et al., 2000; Khisti et al., 2002). This increase in allopregnanolone levels was substantial in male rats, but allopregnanolone was still more than two-fold increased in the estrous phase of female rats (Morrow et al., 2001).

Human studies have been scarce. In female adolescents presenting with acute alcohol intoxication at the emergency room, allopregnanolone and progesterone levels were elevated compared to control patients (Torres and
Ortega, 2003). This elevation in allopregnanolone levels was seen in both phases of the menstrual cycle. Similarly, elevated levels of both allopregnanolone and progesterone were seen in intoxicated males (Torres and Ortega, 2004; however, see Sarkola et al., 1999). After alcohol withdrawal in chronic alcohol users, allopregnanolone levels seems to decrease during the first week of abstinence by 50%, which was significantly more than in controls (Romeo et al., 1996; 2000). Thus far, neurosteroid response to low or moderate doses of alcohol has not been studied in humans.

Alcohol and Cortisol

Long-term alcohol consumption has been considered as a stressor leading to an increased secretion of cortisol (Khisti et al., 2002). However, the effects of acute alcohol intake depends more on the achieved blood alcohol concentration. Acute administration of high doses of alcohol produces an increase in plasma cortisol concentration in both males and females (Jenkins and Connolly, 1968; Aguirre et al., 1995; Inder et al., 1995; Frias et al., 2000; 2002), whereas studies aiming at low blood alcohol concentration (0.25 - 0.75g/kg of body weight) have not revealed any significant changes in cortisol response (Gianoulakis et al., 1996; Holdstock et al., 2005; Pierucci-Lagha et al., 2005; King et al., 2005). However, it might not be the alcohol intoxication in itself that activates the HPA-axis. With intoxicating levels of blood alcohol concentration (34.0 mmol/L) in combination with gastrointestinal symptoms such as nausea and vomiting, the levels of cortisol increased, but in subjects without gastrointestinal symptoms cortisol levels were unaffected (Jenkins and Connolly, 1968).

Because the neurosteroid response to alcohol could be secondary to an HPA-axis activation, the allopregnanolone response to alcohol should be measured together with cortisol levels.
Measuring Alcohol Sensitivity in Humans

Saccadic eye movement parameters are considered a reliable measure of alcohol's effect as they respond to alcohol in a dose-dependent fashion (Katoh, 1988).

A saccade is a rapid eye movement from one target to another that brings the target seen in the periphery on to the fovea. The saccade starts about 180 ms after the target moves and it accelerates rapidly to peak velocity and then decelerates and stops with the fovea on the target.

From a single saccadic eye movement a number of different parameters can be distinguished to determine the degree of pharmacological effect, Figure 4. Saccadic eye velocity (SEV) is the speed by which the eye moves from one target to another. SEV can be an objective measure of CNS depression and the effect of sedative drugs (Hommer et al., 1986; Ball et al., 1991). Several studies have shown that SEV is reduced in a dose-dependent manner by benzodiazepines (Hommer et al., 1986) and this effect is reversed by the benzodiazepine antagonist flumazenil (Ball et al., 1991). Maximum saccadic eye velocity can vary between 200 degrees up to 700 degrees per second. The variability in SEV between individuals is large, but the intra-individual variability, both between test days and within a testing session, is generally low (Gentles and Thomas, 1971; Mercer et al., 1990; Roy-Byrne et al., 1990; Glue, 1991; Sundström and Bäckström, 1998b). SEV increases with the amplitude (size) of the saccade, but reaches its maximum at about 30 degrees of saccade amplitude (Balogh et al., 1975). The saccadic eye velocity is not under voluntary control, and an attempt by the subject to influence the saccade only decreases the saccade amplitude, leaving the velocity/amplitude relationship intact. Neurophysiological data indicates that saccadic eye movements are controlled by the frontal eye fields, substantia nigra, superior collicus, pontine reticular formation, and cerebellum (Becker et al., 1989).

Latency is the time it takes from identification of the new target, decision, and computation, until the initiation of a saccade. It usually varies from 150 to 300 ms and is the time between target movement and saccade onset. Saccade latency is also referred to as reaction time. Latency is often prolonged after sedative drug administration (Konrad, 1991) and increases with age (Carter et al., 1983).

Saccade accuracy is defined as the difference between attempted target and actual target position at the end of the saccade. Usually there is a small undershoot that is corrected by producing a compensatory saccade to reach the attempted target.

Saccade deceleration is the decrease of SEV before stopping at the attempted target. Similarly, saccade acceleration is the increase of SEV after the onset of
the saccade. Some studies have suggested that saccade deceleration is a more sensitive measure of sedative effects than SEV (Figure 4) (Ball et al., 1991).

**Figure 4.** The saccadic parameters that represent a single horizontal saccade. The change in eye position induces a change in potential between the electro-oculography recording electrodes, a change proportional to the displacement of the eyes.

Alcohol decreases SEV (Wilkinson et al., 1974; Baloh et al., 1979; Lehtinen et al., 1979; Jäntti et al., 1983; Stapleton et al., 1986; Moser et al., 1998; Blekher et al., 1999; Ramchandani et al., 1999), Table 2. In most studies, saccade latency is prolonged after alcohol ingestion (Baloh et al., 1979; Levett and Jaeger, 1980; Jäntti et al., 1983); although in some studies, no change was seen (Lehtinen et al., 1979; Holdstock and de Wit, 1999). Saccade accuracy does not seem to be affected by alcohol (Baloh et al., 1979; Lehtinen et al., 1979; Jäntti et al., 1983), Table 2.
Table 2. Alcohol effect on saccadic eye movement parameters.

<table>
<thead>
<tr>
<th>Author</th>
<th>Dose</th>
<th>Adm way</th>
<th>SEV</th>
<th>Latency</th>
<th>Accuracy</th>
<th>BrAC mmol/L</th>
<th>BAC mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilkinson et al., 1974</td>
<td>50g p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>18 – 20.8</td>
<td></td>
</tr>
<tr>
<td>Baloh et al., 1979</td>
<td>0.415g/kg p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.83g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jäntti et al., 1983</td>
<td>Not stated i.v</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>Lehtinen et al., 1979</td>
<td>1.0 g/kg p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>12.1 – 25.1</td>
<td></td>
</tr>
<tr>
<td>Moser et al., 1998</td>
<td>0.5 g/kg p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blekher et al., 1999</td>
<td>Steady state p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Holdstock and de Wit, 1999</td>
<td>0.4 g/kg p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8 g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katoh, 1988</td>
<td>0.43 g/kg p.o</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>von Steveninck et al., 1993</td>
<td>Steady state i.v</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>Blekher et al., 2002</td>
<td>Steady state i.v</td>
<td>↓</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>13-14.1</td>
<td></td>
</tr>
</tbody>
</table>

BrAC, Breath alcohol concentration; BAC, Blood alcohol concentration
AIMS

To investigate if a low dose of a GnRH-agonist is effective for treatment of premenstrual dysphoric disorder.

To test the hypothesis that symptom improvement following low-dose GnRH treatment in PMDD patients is associated with reduced allopregnanolone levels.

To investigate the saccadic eye movement response to a low dose of alcohol in the follicular and luteal phases of the menstrual cycle in women with PMDD and control subjects.

To investigate whether the allopregnanolone response to a low dose of alcohol is different between women with PMDD and controls in the follicular and luteal phases of the menstrual cycle.

To investigate whether there is a difference between healthy women and healthy men in saccadic eye movement measurements and subjective feelings of sedation and intoxication after infusion of a low dose of alcohol.
SUBJECTS AND METHODS

Subjects

In these studies, 65 subjects were included. In the low-dose GnRH clinical trial (Paper I), PMDD patients were recruited at the out-patient wards of four hospitals in Sweden: Department of Obstetrics and Gynecology at Eskilstuna Hospital, Umeå University Hospital, Lycksele Hospital, and Uppsala University Hospital. Paper II was a sub-study of the low-dose GnRH clinical trial and consisted of those women with PMDD that were included at the Department of Obstetrics and Gynecology in Umeå. All trials of papers II to V were performed at the Department of Obstetrics and Gynecology, Umeå University Hospital, Umeå. Healthy female control subjects and healthy men were recruited through advertisement in the local paper and by posters at the hospital.

The study procedures were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki. The patients all gave informed consent and the studies were approved by the Ethics Committee of Umeå University, Sweden. The low-dose GnRH trial was also approved by the Medical Products Agency, Sweden.

PMDD Diagnosis

Independent of the terminology used in the different papers (according to the suggestions by the reviewers), PMDD diagnoses were confirmed in the same way throughout the studies although the description in paper IV is different. The diagnosis was based on daily prospective symptom ratings on the Cyclicity Diagnoser (CD) scale during two menstrual cycles prior to inclusion. The CD scale is an instrument designed for diagnosing cyclical symptoms and was validated for diagnosis of premenstrual syndrome in Paper I by asking twenty-three patients before inclusion to keep duplicate records of their daily symptoms during one menstrual cycle, using both the CD scale and a previously validated visual analog scale (VAS) (Sanders et al., 1983; Hammarbäck et al., 1989b). The CD scale consists of four negative mood parameters (depression, fatigue, irritability, and tension), three positive mood parameters (cheerfulness, friendliness, and energy), and four somatic symptoms (headache, swelling, breast tenderness, and menstrual bleeding). In addition, the CD scale contains a score for measuring daily social functioning
and work performance. The rating scale for impact on daily life has 8 scale steps.

0 - no impact on daily life
1 - patient notices
2 - family notices
3 - impaired family relations
4 - avoiding social relations
5 - refrain from social relations
6 - difficulties working
7 - can’t work
8 - can’t go to work

The CD scale is a Likert scale with a sensitivity to detect a difference in mood of one scale step (Seippel et al., 1998). In Papers I and II, the scale was graded from 1 to 9, where 1 represented complete absence of a specific symptom and 9 represented the maximal severity of the symptom. In Papers III to V, the grading was changed to 0 to 8, with the same endpoints as the previous scale. This change was made due to input from the participating women because they considered it would be more logical to grade complete absence of a symptom as 0 rather than 1.

Patients were considered to have PMDD if they had a significant increase in at least five symptoms (four negative mood symptoms and one physical symptom) during 9 days preceding onset of menstrual bleeding compared to 9 mid-follicular days (Hammarbäck et al., 1989b). The definition of symptom change from mid-follicular to the late luteal phase was based on individual statistical tests. In Paper I-III symptom scores for each symptom from 9 mid-follicular days were compared with 9 late-luteal phase days using the Wilcoxon Matched-Pairs Signed–Ranks Test, and a p-value less than 0.05 was required for each individual symptom. In Paper IV patients were considered to have PMDD if they displayed a 100% increase in at least five symptoms during 5 premenstrual days compared to 5 postmenstrual days. This change in diagnostic procedure compared to previous papers was made because of a reviewer’s request but did not change the diagnosis in our patient group.

PMDD also had to be associated with a clinically significant social and occupational impairment. The threshold for severity was a score of 2 (0 - 8 scale) or more for more than 3 days during the luteal phase (family notices the patient’s symptoms). At least 1 week of sparse symptoms with scores less than 2 in the mid-follicular phase were required. Patients with current mental disorder or a history of drug abuse, as determined by careful medical history, were also excluded from the studies.

Example of prospective ratings on the CD scale is given in Figure 5.
Figure 5. Example of daily prospective ratings in PMDD patients and control subjects, in this case from Paper III. Mean ± SEM daily symptom ratings on a 9-point CD scale of irritability, depressed mood, and tension and effect on daily life in 12 women with and without PMDD. The cycles represent an ideal 28-day cycle with 14 postmenstrual days and 14 days preceding onset of menstrual bleeding.

Paper I - II
This study screened 40 healthy women between 25 and 45 years of age who had suffered from premenstrual mood changes for more than six months and were seeking treatment from a gynecologist. Of these, 31 women fulfilled criteria for PMDD and were enrolled in the study. Exclusion criteria included the following: ongoing treatment with oral contraceptives, other steroid hormones, benzodiazepines, or antidepressants. In addition, women with irregular menstrual cycles (e.g., variation of more than 28 ± 3 days) were not included. Twenty-seven PMDD patients completed the low-dose GnRH clinical trial.

The study group in Paper II was part of the low-dose GnRH agonist clinical trial and was carried out only at the department of Obstetrics and Gynecology, Umeå. Of 18 patients with PMDD included in this study, 12 patients had blood samples taken within the stipulated time frames and were included in further analyses.
Table 3. Demographic data for the study group in Paper I, (n = 27). Data presented as Mean ± SD.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (year) (SD)</td>
<td>37.6 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Parity, (number) (SD)</td>
<td>1.9 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Education university/college, n</td>
<td>10 (37%)</td>
<td></td>
</tr>
<tr>
<td>Full time employment, n</td>
<td>16 (59%)</td>
<td></td>
</tr>
<tr>
<td>Married, n</td>
<td>18 (67%)</td>
<td></td>
</tr>
<tr>
<td>Mother and/or sisters with PMS, n</td>
<td>18 (67%)</td>
<td></td>
</tr>
<tr>
<td>Previous psychiatric treatment, n</td>
<td>12 (44%)</td>
<td></td>
</tr>
<tr>
<td>Previous post-partum depression, n</td>
<td>3 (11%)</td>
<td></td>
</tr>
</tbody>
</table>

Papers III - V
Fourteen women with confirmed PMDD were included in the study. Patients were between the ages of 25 and 45, had regular menstrual cycles, and had sought treatment at the out-patient gynecology clinic (Umeå University Hospital) for severe dysphoric premenstrual changes of more than six months. In addition, twelve physically healthy women and twelve physically healthy men between the ages of 20 and 45 were recruited for the studies in Papers III - V. Healthy female control subjects had regular menstrual cycles (28 ± 3 days) and no significant dysphoric symptoms during the premenstrual period on prospective ratings using the CD scale. Exclusion criteria for all groups were ongoing treatment with oral contraceptives, other steroid hormones, benzodiazepines, or other psychotropic drugs. Subjects with a history of mental disorders during the past 2 years were excluded from the study. The measure of recent drinking history was a retrospective report of the total amount of alcohol consumed during the last four weeks preceding the inclusion of the study. Only light and moderate consumers (intake of less than 25 g of alcohol per day) were included.

Table 4. Demographic data of the study groups in Papers III-V. Data presented as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Healthy women n = 12</th>
<th>PMDD patients n = 14</th>
<th>Healthy men n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>29.9 ± 5.6</td>
<td>35.1 ± 4.9</td>
<td>30.0 ± 6.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67.3 ± 17.8</td>
<td>70.6 ± 13.0</td>
<td>77.2 ± 7.1</td>
</tr>
<tr>
<td>Married, n</td>
<td>7 (58%)</td>
<td>10 (71%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>Employed, n</td>
<td>12 (100%)</td>
<td>14 (100%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Education university/college, n</td>
<td>10 (83%)</td>
<td>8 (57%)</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Psychiatric history, n</td>
<td>1 (8%)</td>
<td>6 (43%)</td>
<td>0</td>
</tr>
<tr>
<td>Mean alcohol consumption, g/week</td>
<td>21.8 ± 14.2</td>
<td>15.5 ± 12.8</td>
<td>54.1 ± 49.1</td>
</tr>
</tbody>
</table>
Experimental Design

Papers I - II

The low-dose GnRH agonist trial was a randomized, double-blinded, and placebo-controlled cross-over study. The patients were randomized to either intranasal GnRH-agonist (buserelin) in a dosage of 100 µg once daily for two months or placebo for two months before a cross-over was made to the other treatment (Figure 6). The main outcome measure was the daily symptom ratings on the CD scale that patients kept throughout the study.

![Figure 6. Schematic diagram of the low-dose GnRH study.](image)

Progesterone, estradiol, LH, and FSH were analyzed every second week. The blood sampling was aimed at the mid-follicular phase (1 week after start of bleeding) and luteal phase (1 week before start of bleeding). For Paper II, these blood samples were used for analyses of progesterone, allopregnanolone, and pregnanolone. However, only blood samples taken during ovulatory cycles (serum progesterone levels ≥ 15 nmol/L) and within a stipulated time frame (day 1 to day 9 in the luteal phase) were used for the study. Among the 18 patients with PMDD that were recruited for the study, 12 patients had 24 menstrual cycles that fulfilled these criteria. Data for adverse reactions and well-being were collected at each visit.

Papers III – V

Saccadic eye movement measurements were made on four occasions during the menstrual cycle: twice in the mid-follicular phase (6-12 days after the onset of menstrual bleeding) and twice in the late luteal phase (1-7 days prior to the onset of menstruation), Figure 7. Within each cycle phase, an alcohol and a placebo infusion was given in a randomized order with an interval of 48 hours. Male participants made saccadic eye movement measurements on two occasions, with 48-hours interval. As in the female group, alcohol and placebo
was given in a randomized order. Because of technical problems, we could not obtain any SEV recordings from two PMDD patients, which is why 12 PMDD patients are included in Paper III and 14 PMDD patients are included in Paper IV. No subjects consumed alcohol within 24 hours of the test sessions and were furthermore encouraged not to consume alcohol during the study period.

**Figure 7.** The timing of alcohol/placebo challenges in Papers III – V (female subjects) in relation to the physiological estradiol and progesterone levels across the menstrual cycle.

An intravenous cannula was inserted in each forearm and blood samples were taken for baseline levels of estradiol and progesterone in plasma. To establish baseline, three sets of SEV measurements and visual analogue ratings of sedation and alcohol intoxication were made with 5 minutes rest in between.

Thereafter, a double-blinded randomized intravenous infusion of either placebo or alcohol was given for 30 minutes. The experimental medications were prepared by the University Hospital Pharmacy. The alcohol solution contained 10% of alcohol and was dissolved in NaCl (9mg/ml, Baxter Healthcare Corporation, IL USA). Each patient received 0.2 g/kg alcohol and the infusion rate was adjusted accordingly. The placebo preparation consisted of NaCl. In order to give a slight odor of alcohol, a small patch of cotton soaked in alcohol was placed near the subject’s head.

After the start of the alcohol infusion, SEV recordings and visual analogue ratings were made at 5, 15, 25, 35, 45, 55, 65, and 75 minutes, but only the time points 5, 15, 25, and 35 minutes were used in the analysis in Paper III. For Paper V, time points 5, 15, and 25 minutes were used in the analysis. Blood samples for alcohol levels were taken at 5 and 25 minutes after the start of the infusion.
In Paper IV, blood samples for serum allopregnanolone and serum cortisol levels were drawn at baseline and 25, 55, and 75 minutes after the start of the infusion.

**Measurements of Saccadic Eye Movements**

**Papers III and V**

Saccadic eye movement parameters were measured using electrooculography (EOG) with the Cardiff Saccade Generation and Analysis System (CSGAAS5); this is fully documented elsewhere (Marshall et al., 1985; Marshall and Richens, 1989), Figure 8. Parameters measured in these studies were SEV, saccade acceleration, saccade deceleration, saccade accuracy, and saccade latency.

The test was performed in a quiet, semi-lighted room with the patient sitting in a comfortable chair. A pillow placed next to the head prevented head movement. EEG cup electrodes (Synetics AB, Stockholm, Sweden) with a small amount of electrode-gel (Elefix, Nihon Kohden) were used. After the skin had been exfoliated with Skinpure cream (Nihon Kohden), the electrodes were placed one cm lateral of the outer canthus of both eyes, with one common electrode in the center of the forehead. Electrode impedances was measured and confirmed to be less than 5 kohm.

![Figure 8. Diagram of the experimental arrangement for saccadic eye movement measurements.](image_url)
The subject was instructed to watch an array of light-emitting diodes (LED), placed at eye-level, 67 cm from the glabella. The subject was asked to look at the illuminated LED (the target) and to move her eyes to the next target (the next illuminated LED) as that LED was turned off and the next one in the array was lit. Subjects were instructed not to anticipate targets. The target movements took place at 1.5-sec. intervals. A fixed, non-random sequence of 4 x 24 targets, producing target steps of 10, 20, 30, and 40 degrees, was displayed with a brief rest in between. The first four of these 24 target steps of each session were not included in the subsequent analyses to allow the subject to adjust to the procedure. The EOG was DC amplified and low-pass filtered (-3dB at 50 Hz) before being digitized to 12-bit resolution at a sampling frequency of 250 Hz. A personal computer controlled the target movements and digitized the waveform using an analogue-digital converter.

The 80 individual EOGs, resulting from the 4 x 20 target steps, were stored and analyzed off-line according to the method used by Marshall and Richens (Marshall and Richens, 1989). First, the digitized data from each target displacement was processed to locate saccades. To avoid preemptive saccades and blinking artifacts, only saccades initiated between 50 – 400 milliseconds after target movement were included. To be considered a saccade, the recorded eye movement also had to display a velocity of more than 100 degrees/second. Second, each saccade was analyzed to determine the size of the saccade in degrees, the peak saccadic velocity, the peak saccade deceleration, and the latency from target movement to onset of saccade. Saccade accuracy was determined by comparing the actual eye position at the end of the saccade with the attempted target.

SEV, saccade acceleration and saccade deceleration were further processed by plotting a velocity-saccade size curve, known as the main sequence (Baloh et al., 1975). The relationship between saccade size and peak velocity is important because it remains intact even when voluntary control of saccades is attempted. The main sequence was fitted by a quadratic equation to the peak velocity data using the calculated saccade angle as the independent variable. The influence of outliers in the data was minimized by carrying out the fitting procedure twice and weighing the second fit with the inverse of the square of the residuals from the first fit. The values of peak velocity for 10-, 20-, 30-, and 40-degree saccades were then calculated by interpolation. Saccades with amplitude of 30 degrees were chosen for further analyses as SEV reaches a maximum at approximately 30 to 35 degrees of angular movement (Baloh et al., 1975).
Visual Analogue Ratings of Sedation and Intoxication

Papers III - V
A visual analogue score scale (VAS) was used to rate subjective feelings of sedation and intoxication during the alcohol and placebo challenges. The scale measured from 0 to 100 mm where 0 equaled complete absence of sleepiness/intoxication and 100 represented falling asleep/heavy intoxication. The patients were asked to rate their feelings of sedation and alcohol intoxication using the VAS scale after every set of saccadic eye movement measurements.

Steroid Assays

Paper I
Progesterone concentrations were measured using solid-phase chemiluminescent immunoassay (Immulite, Diagnostic Products Corporation, (DPC) USA). The limit of quantification was 0.6 nmol/L with intraassay and interassay coefficients 7.3% and 3.6%, respectively. Total plasma estradiol-17β was measured using chemiluminescent immunoassay (Diagnostic Products Corporation, USA). The limit of quantification was 44 pmol/L with the intraassay and interassay coefficients 6.5% and 8.6%, respectively. LH and FSH concentrations were measured using microparticle enzyme immunoassay (Abbot Laboratories, IL; USA). The assay sensitivity for LH was 0.5μIU/L with intraassay and interassay coefficients 7.8% and 7.0%, respectively. FSH assay sensitivity was 0.37 IU/L with intraassay and interassay coefficients 3.5% and 2.3%, respectively.

Paper II
Allopregnanolone was measured using radioimmunoassay (RIA) after diethylether extraction and HPLC purification of samples. Recovery was determined for each assay using 300-500 cpm of tritium-labeled allopregnanolone, [9,11,12-3H(N)]-5α-pregnan-3α-ol-20-one (Perkin Elmer Life Sciences, Boston, USA) added to a plasma sample before extraction and by measuring the amount recovered after HPLC. The recovery of allopregnanolone averaged 98% and the results are compensated for recovery. All samples were analyzed using a polyclonal rabbit antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled to bovine serum albumin (a gift from Dr. Robert H Purdy, Department of Psychiatry, College of Medicine, University of California, San Diego). The antiserum was used in a dilution of 1/5000 and the antibody solutions were prepared in the same way as described earlier by Timby et al. (2005). The sensitivity of the
assay was 25 pg with an intraassay coefficient of variation for allopregnanolone of 6.5% and an interassay coefficient of variation of 8.5%. Pregnanolone was measured by radioimmunoassay (RIA) after diethylether extraction and HPLC purification of samples (Sundström et al., 1998a). Briefly the antiserum was raised against 3α, 21-dihydroxy-5β-pregnan-20-one 21-hemisuccinate coupled to bovine serum albumin in a rabbit by Dr Robert H Purdy. Antibody was used in a dilution 1:2300, and the solution was prepared using [11,12] 3H-pregnanolone custom synthesized by New England Nuclear (NEN). The recovery of pregnanolone was 93%. The results are compensated for recovery. The sensitivity of the assays was 25 pg, with an intraassay coefficient of variation of 6.5% and interassay coefficient of variation of 8.5%. Measurements of plasma progesterone were made using Delfia progesterone kits (Wallac Oy, Turku, Finland), a fluoroimmunoassay, according to the manufacturer’s instructions.

The reference menstrual cycles are described in detail in Paper II.

Papers III - V

Progesterone and estradiol were analyzed on Immulite (DPC, Los Angeles, CA, USA). For the estradiol assay, the measure interval was 73 - 7300 pmol/L. Levels below 73 pmol/L were stated as < 73 pmol/L. Estradiol total coefficient of variation was 11.1% at 194 pmol/L and 10.8% at 531 pmol/L. Progesterone total coefficient of variation was 16.5% at 3.06 nmol/L and 6.7% at 71.2 nmol/L.

Allopregnanolone was measured with radioimmune assay (RIA) after pre-assay diethylether extraction and celite chromatography purification as described previously (Bäckström et al.,1986; Corpechot et al., 1993; Andréen et al., 2005). [11,12]3H-allopregnanolone (NEN, Boston, USA) was used for a tracer and for internal recovery estimates in the assays. The allopregnanolone antiserum was raised against 3α-hydroxy-20-oxo-5α-pregnan-11α-yl carboxymethyl ether coupled to bovine serum albumin. The intraassay coefficient of variation was 6.5% and interassay coefficient of variation was 8.5%.

Serum concentration of cortisol was measured by solid phase fluoroimmunoassay (Delphia, Wallac Oy, Turku, Finland). The cortisol assay sensitivity was 15 nmol/L. The intraassay coefficient of variation was 4.5% at 230 nmol/L and interassay precision was 3.5% at 230 nmol/L.

Alcohol in serum was determined by gas-liquid chromatography on GLC 8600 (Perkin Elmer, USA) at the department of Clinical Chemistry, Norrlands University Hospital, Umeå, Sweden. The between day coefficient of variation was 2.1% at 10.5 and 31.0 mmol/L.
Statistics

The statistical procedures are described in detail in each paper.
In Paper I, analysis was made using two-way ANOVA with repeated measures. Estradiol and progesterone serum concentrations were transformed to standard scores (z-score).
In Paper II, negative mood symptoms (i.e., tension, irritability, and depressed mood) were grouped together as mean scores of summarized symptoms. ANOVA with repeated measures was used to distinguish possible difference between luteal phase ratings and type of treatment. Allopregnanolone and progesterone serum concentrations were transformed to standard scores (as in Paper I). Hormone levels between buserelin responders and placebo responders were compared using Mann-Whitney U-test and between treatments in each group by Wilcoxon Matched-Pair Signed-Rank Test.
In Paper III, two-way ANOVA with repeated measures was used to determine which variables were affected by alcohol and to evaluate the phase and group effects on saccade parameters. One-way ANOVA was used to compare alcohol serum concentrations, SEV, and saccade deceleration between groups and cycle phases at the 5- and 25-minutes time points.
For Paper IV, sedation and intoxication scores as well as allopregnanolone and cortisol serum concentrations were calculated as delta scores. For cortisol analyses this was especially important as measurement were not standardized with respect to diurnal changes in cortisol levels. Four-way ANOVA with repeated measure was used to evaluate the alcohol effect on the VAS scores and allopregnanolone and cortisol levels. Follow-up analyses by two-way ANOVA and post hoc test (Tukey Honestly Significant Difference test) were made if there were any indication of significant interactions.
In Paper V, sedation and intoxication scores and saccade parameters were calculated as delta scores. Data from follicular and luteal phase were combined in the female group. Mann-Whitney U test was used to compare baseline values of saccade parameters and VAS score ratings between males and females. Three-way ANOVA with repeated measure was used to evaluate the alcohol-induced impairment in saccade parameters and VAS score ratings and menstrual cycle effect. Blood alcohol concentrations were evaluated using two-way ANOVA with repeated measures. Correlations were made using Pearson’s correlation.
Statistical analyses were performed using the standard statistical package SPSS, version 10.0 and 11.5. A p-value of < 0.05 was considered as statistical significant.
RESULTS

The Effect of Low-dose GnRH-agonist for Treatment of PMDD, (Paper I)

Treatment with low-dose buserelin induced a significant improvement in premenstrual depression and irritability scores compared to placebo treatment, \( p < 0.01 \) and \( p < 0.05 \), respectively (Figure 9). Also, PMDD patients indicated higher scores for cheerfulness and friendliness during buserelin treatment than during placebo, \( p < 0.01 \). Some of the somatic symptoms were also improved by buserelin treatment; a significant relief was noted in headache (\( p <0.01 \)) and swelling (\( p < 0.05 \)), while breast tenderness was unaffected.

![Figure 9. Daily symptom ratings on a 9-point CD scale of irritability, depressed mood, and tension during the pre-, the placebo-, and the buserelin-treatment cycles. The cycles represent an idealized 28-day cycle. Data were centered on the first day of menstrual bleeding. Each point represents the group mean. Compared to placebo, buserelin significantly relieved premenstrual depressed mood scores (\( p < 0.01 \)) and irritability scores (\( p < 0.05 \)). Error bars are not displayed for clarity reasons.](image)
Because no wash-out phase was included in the study design, concerns about spill-over effects were also addressed in the analyses. Women who started with placebo reported significantly lower symptom scores of irritability, depression, friendliness, and cheerfulness after the cross-over to buserelin, indicating an effect attributable to the buserelin treatment. In addition, somatic symptoms – such as breast tenderness ($p < 0.033$), swelling ($p < 0.015$), and headache ($p < 0.018$) – were significantly improved after cross-over to buserelin treatment. In women who started with the buserelin treatment, depression was the only symptom that deteriorated after the cross-over to placebo treatment. These findings indicate that some of the positive effects of buserelin treatment could have been obscured because of spill-over effects from active treatment during the placebo period.

Although buserelin treatment induced a significant improvement in symptom ratings, the question remained whether this was solely attributable to the effect of anovulation. In Table 5, symptom scores during ovulatory buserelin and placebo cycles were compared between treatments in 22 PMDD patients who had at least one ovulatory cycle during buserelin treatment. During buserelin treatment, significant improvement in the individual symptoms was noted in depressed mood (Table 5).

**Table 5.** Mean ± SEM scores for mood and somatic symptoms during placebo and buserelin treatment during ovulatory cycles.

<table>
<thead>
<tr>
<th></th>
<th>Placebo n = 22</th>
<th>Buserelin n = 22</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed mood</td>
<td>3.39 ± 0.4</td>
<td>2.76 ± 0.3</td>
<td>0.049</td>
</tr>
<tr>
<td>Irritability</td>
<td>3.52 ± 0.4</td>
<td>3.28 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>Tension</td>
<td>2.66 ± 0.3</td>
<td>2.41 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>Happy</td>
<td>5.12 ± 0.3</td>
<td>5.58 ± 0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Friendly</td>
<td>5.21 ± 0.4</td>
<td>5.79 ± 0.3</td>
<td>0.062</td>
</tr>
<tr>
<td>Energy</td>
<td>5.14 ± 0.4</td>
<td>5.36 ± 0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Swollen</td>
<td>3.01 ± 0.4</td>
<td>3.01 ± 0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Breast tension</td>
<td>2.65 ± 0.4</td>
<td>3.11 ± 0.5</td>
<td>ns</td>
</tr>
<tr>
<td>Headache</td>
<td>2.14 ± 0.3</td>
<td>1.66 ± 0.2</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Adverse effects from treatment mainly resided from ovulatory disturbances. The low-dose buserelin treatment increased cycle length significantly. Fifteen patients (56%) had at least one anovulatory cycle during buserelin treatment. Five patients (19%) became anovulatory throughout the whole period of active treatment. Those patients who had anovulatory cycles at some point were
significantly older than those who maintained regular cycles. Eleven patients (41%) experienced prolonged menstrual cycles (> 35 days) at some time during the study. Nine patients (33%) experienced spotting at some point during buserelin treatment.

Steroid Responses to Low-dose GnRH agonist Treatment and Placebo, (Paper II)

Based on the scores of daily life impairment and summarized negative mood scores during the diagnostic cycles and during placebo treatment, the women were divided into two groups, buserelin responders and placebo responders. Of the 12 patients included in the study, six were buserelin responders, whereas the remaining six were placebo responders.

Placebo Responder Group
Placebo responders reported a significant improvement in summarized negative mood and daily life impairment with both the placebo treatment (p < 0.01 and p < 0.05, respectively) and buserelin treatment (p < 0.01 and p < 0.05, respectively) compared to pretreatment.

During placebo treatment the placebo-responders had lower z-scores of allopregnanolone compared to what was seen in the buserelin responders during placebo (p < 0.05), Table 6.

Buserelin Responder Group
The buserelin responders reported a significant improvement following buserelin treatment in summarized negative mood symptoms and daily life impairment compared to both placebo treatment (p < 0.05 and p < 0.001, respectively) and pretreatment (p < 0.01 and p < 0.01, respectively). The symptom improvement during buserelin treatment was accompanied by lower progesterone (p < 0.05) and allopregnanolone z-scores (p < 0.05) compared to during placebo treatment. There was no difference in steroid concentrations that were not normalized between treatments, Table 6.

During buserelin treatment there were no differences in neurosteroid or progesterone concentrations or normalized z-score values between buserelin responders and placebo responders.
Table 6. Mean ± SD of progesterone and neurosteroid levels during the late luteal phase of placebo and buserelin treatment in placebo-responders and buserelin responders.

<table>
<thead>
<tr>
<th>Hormone/neurosteroid</th>
<th>Buserelin responders</th>
<th>Placebo responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>placebo mean ± SD</td>
<td>buserelin mean ± SD</td>
</tr>
<tr>
<td></td>
<td>placebo mean ± SD</td>
<td>buserelin mean ± SD</td>
</tr>
<tr>
<td>Progesterone, z-score</td>
<td>0.39 ± 0.7</td>
<td>-0.94 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Progesterone, nmol/L</td>
<td>28.0 ± 5.0</td>
<td>17.8 ± 2.9</td>
</tr>
<tr>
<td>Allopregnanolone, z-score</td>
<td>0.34 ± 0.2</td>
<td>-0.37 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Allopregnanolone, nmol/L</td>
<td>1.4 ± 0.3</td>
<td>0.99 ± 0.3</td>
</tr>
<tr>
<td>Pregnanolone, nmol/L</td>
<td>0.83 ± 0.3</td>
<td>0.68 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly lower compared to placebo cycle (p < 0.05) Wilcoxon Matched-Pairs Signed-Ranks Test.

<sup>b</sup>Significantly lower compared to placebo cycle of buserelin responders (p < 0.05) Mann-Whitney U-test.

Effects of Alcohol on Saccadic Eye Movement in Women with PMDD and Healthy Women, (Paper III)

Alcohol Response on Saccadic Eye Movements

Alcohol concentrations at the 5-minute and 25-minute time points were similar between groups and cycle phases. Compared to placebo, the alcohol infusion induced a marked decrease in SEV (p < 0.01), and marked increases in saccade deceleration (p < 0.001) and subjective alcohol intoxication scores (p < 0.001). There was a marked initial effect after 5 minutes of alcohol infusion in both SEV (p < 0.001) and saccade deceleration (p < 0.001) in both groups and cycle phases.

Alcohol Response Between Cycle Phases

Women with PMDD displayed blunted SEV and saccade deceleration responses (p < 0.01 and p < 0.01, respectively) to alcohol infusion in the late luteal phase compared to the mid-follicular phase, indicating a decreased sensitivity to the low dose of alcohol in the luteal phase, Figure 10. Control subjects did not change their SEV or saccade deceleration responses to alcohol between cycle phases.
Figure 10. Mean ± SEM of change in SEV, saccade deceleration, and alcohol intoxication scores from pre-infusion baseline in response to alcohol infusion in control subjects and PMDD patients during the mid-follicular phase and late luteal phase of the menstrual cycle.

Alcohol Response Between Groups

There was no difference between patients with PMDD and control subjects in alcohol-induced SEV or saccade deceleration response in either cycle phase.
The Effect of Alcohol on Allopregnanolone and Cortisol Serum Concentrations, (Paper IV)

Compared to controls, PMDD patients had lower baseline serum concentrations of allopregnanolone in the mid-follicular phase ($p < 0.01$) and in the late luteal phase ($p < 0.05$).

In the luteal phase, the allopregnanolone response to alcohol infusion was significantly greater compared to the placebo infusion. Allopregnanolone levels declined significantly following alcohol rather than placebo at the 25-minute ($p < 0.01$), 55-minute ($p < 0.01$), and 75-minute time points ($p < 0.05$) after the start of the infusions, Figure 11. There were no differences between PMDD patients and control subjects in alcohol-induced effects on allopregnanolone serum concentrations.

**Figure 11.** Mean ± SEM of allopregnanolone and cortisol serum concentrations in response to alcohol and placebo infusion during the follicular phase (a) and the late luteal phase (b) of the menstrual cycle. The alcohol infusion was 0.2 g/kg. The gray horizontal bars indicate the infusion time.

* $p < 0.05$, difference from baseline, ** $p < 0.01$, difference from baseline, *** $p < 0.001$, difference from baseline, # $p < 0.05$, difference from placebo, ## $p < 0.01$, difference from placebo.
Cortisol serum concentrations declined during both the alcohol and placebo infusion, but no difference in cortisol response to alcohol or placebo was noted, Figure 11. Likewise, there were no differences between PMDD patients and controls in alcohol-induced effects on cortisol serum concentrations.

The Effect of Alcohol on Saccadic Eye Movement in Men and Healthy Women, (Paper V)

Blood alcohol concentrations at the 5-minute and 25-minute time points were similar between groups. Based on prior findings of no difference between cycle phases in saccadic eye movement sensitivity to alcohol among healthy women, data from the follicular and luteal phases were combined in the female group (Paper III).

Compared with placebo, the alcohol infusion induced a marked increase in subjective feeling of intoxication in both males and females \( (p < 0.001) \). Only women reported an increase in saccade accuracy and in self-rated intoxication scores already at 5 minutes of alcohol infusion. Between 5 and 25 minutes of alcohol infusion both groups displayed unaltered SEV and saccade deceleration responses, while saccade accuracy and intoxication were further deteriorated.

No significant differences were seen between male and female subjects in response to the alcohol infusion in any of the saccade parameters or VAS scorings, Figure 12.
Figure 12. Mean ± SEM of changes in SEV, saccade accuracy, saccade deceleration, and alcohol intoxication scores from pre-infusion baseline in response to alcohol infusion in healthy female (n = 12) and male subjects (n = 12). The alcohol infusion was 0.2 g/kg and lasted for 30 min, and gray horizontal bars in the figures indicate its time course. No difference was detected between male and female subjects in alcohol-induced SEV, saccade deceleration, or saccade accuracy response.
DISCUSSION

Low-dose GnRH Agonist for Treatment of PMDD

Buserelin, a GnRH analogue, in low dose can reduce premenstrual depression and irritability. Compared to the placebo, buserelin induced a significant improvement in both positive and negative psychological symptoms during the premenstrual week. Somatic symptoms, such as premenstrual headache and swelling, were also improved, whereas buserelin did not affect breast tenderness scores. This finding agrees with other studies examining the effect of various regimens of GnRH treatment in short- (Hammarbäck et al., 1988) or long-term treatment courses (Mortola et al., 1991; Mezrov et al., 1994). However, the primary goal of previous studies has been to induce an anovulatory state that lowers gonadal hormones. The effect of anovulation as a symptom-relieving factor in PMDD has been firmly established, and during spontaneous as well as GnRH agonist-induced anovulation the cyclicity of premenstrual symptoms disappeared (Hammarbäck et al., 1988; 1991; Mortola et al., 1991; Mezrov et al., 1994; Brown et al., 1994; Freeman et al., 1993; Studd and Leather, 1996). In contrast, the low dose of buserelin that was used in the current study proved to be efficient although more than half of the cycles were ovulatory with estradiol levels clearly not suppressed.

When only ovulatory cycles were analyzed the symptom improvement with low-dose GnRH was less impressive. The only symptom that was improved during ovulatory buserelin cycles was depressive mood. Although this could indicate that the effect of low dose GnRH is mainly due to anovulation, it could also be a result biased by low power. Five subjects were anovulatory throughout the entire buserelin treatment period and in other subjects these analyses are based on only one (instead of two) treatment cycles.

The treatment dose of GnRH agonist used in the present study requires a clarification. In gynecologic practice, GnRH agonists can be used to treat estrogen dependent conditions such as endometriosis and uterine fibroids. The recommended daily dose for treatment of endometriosis with nasal GnRH agonist (Suprefact 100μg/dose) is 900 μg.

Evidently, a carry over effect from buserelin treatment to placebo treatment was present among those women who started with buserelin. This carry-over effect particularly affected the somatic symptoms (swelling and breast tenderness) that remained indifferent after the cross-over from buserelin to placebo. Similarly, irritability scores and positive mood scores were unaltered after cross-over in women who started with active treatment. Women who
started with placebo treatment displayed significant improvements in negative mood symptoms, positive mood symptoms, and somatic symptoms after the cross-over to buserelin. These findings indicate that part of the difference between placebo and buserelin treatment might have been obscured by an extended treatment effect of buserelin during the placebo cycles among patients starting with buserelin.

Clearly, if a more extensive washout period had been included between the two treatments, a more impressive effect of low-dose GnRH could have been demonstrated. While preparing the study, we assumed that no washout would be needed because the effect of treatment only was evaluated during the last seven days of each menstrual cycle.

One disadvantage of buserelin treatment was its tendency to induce anovulation in spite of the low dose that was used. Fifty-six percent of patients had at least one anovulatory cycle during the study and 19% of the patients became completely anovulatory throughout the treatment period. The unexpectedly high rate of anovulatory cycles with irregularities in the cycle length after buserelin treatment was also a limitation to the study because it made it difficult to estimate the optimal time schedule for blood sampling. A more frequent blood sampling would have made it easier to capture the hormonal change and to fully assess corpus luteum function.

Blood samplings were originally scheduled to be performed during mid-follicular phase and mid-luteal phase, but during unexpectedly short as well as prolonged cycles we were unable to maintain this schedule. Given this limitation, it is possible that the number of anovulatory cycles might have been underestimated and that the true number of anovulatory cycles caused by buserelin treatment is even higher.

The incidence of anovulation depended on age because women with at least one anovulatory cycle were significantly older compared to those who maintained regular cycles. This finding indicates that 100 μg of buserelin might be too high a dosage for women approaching their perimenopausal period. Irrespective of age, patients receiving low-dose GnRH agonist therapy should be monitored to ascertain that long periods of amenorrhea do not occur.

Today, GnRH agonists are not the first choice of treatment for PMDD. Apparently, a dose as low as 100 μg increases the risk of anovulatory cycles and the risk for long-term negative side effects (such as bone demineralization and endometrial hyperplasia) is imminent. Possibly, an even lower dose than 100 μg would minimize the number of anovulatory cycles, but to date no such
drug is on the market. An alternative approach would be to evaluate ordinary (full-dose) GnRH agonist treatment in combination with low-dose HT. Thus far, studies examining add-back with estradiol and progestagens to GnRH agonist treatment have employed 2 mg estradiol doses in combination with 10 mg progestagens during the last 14 days of the treatment cycle. This regimen is more than sufficient to treat the vegetative symptoms that are induced by the GnRH treatment, but will result in the return of the premenstrual symptoms, at least to some degree. Lower doses of estradiol/progestagen add-back might be more beneficial for PMDD patients, especially in the light of studies indicating that estradiol might be as symptom provoking as progesterone in PMDD patients (Schmidt et al., 1998). The difficulty in this case would be to find the threshold level for avoiding loss of bone mineral density and minimizing vasomotor symptoms and still relieve premenstrual symptoms.

Given the limited evidence for low-dose GnRH agonist, first-line treatment for severe premenstrual symptoms is still serotonin reuptake inhibitors. The documentation of the beneficial effect from SSRI treatment is substantial (Freeman et al., 1999; Pearlstein et al., 2000; Halbreich et al., 2002; Wyatt et al., 2002). Although many women are reluctant to use antidepressive drugs for their PMDD, the effect on both mood and physical symptoms is evident. An intermittent dosage during the luteal phase only has been proven to be as efficient, or even more efficient, than continuous treatment (Sundblad et al., 1993; Halbreich et al., 1997; Wikander et al., 1998). By using intermittent dosing, it is possible to limit the number of days with decreased libido, which in turn could improve tolerability for the women.

Steroid Response to GnRH Agonist and Placebo Treatment

Our main finding was that a decrease in symptom severity was accompanied by decreased allopregnanolone serum concentrations when investigated in individual patients. Compared to pretreatment cycles, summarized negative mood symptom (depressed mood, tension, and irritability) severity and impairment on daily life was improved with both low-dose buserelin and placebo. Among buserelin responders, the allopregnanolone level decreased in parallel with the decrease in the symptom severity during buserelin treatment. Among placebo responders, the allopregnanolone concentration during the placebo treatment was significantly lower than what was seen among buserelin responders during placebo treatment. Allopregnanolone levels in placebo responders were similar during the placebo and GnRH treatment.
All women in this substudy had a progesterone serum level of 15nmol/L or more indicating that they were all ovulating. It is possible that women receiving a low dose of GnRH agonist treatment with somewhat down regulated ovarian function experience improvement in symptoms as a result of a decline in allopregnanolone levels and that the levels in these women are lower than the peak symptom inducing concentration. Although our findings are preliminary due to the small sample size, decreased level of allopregnanolone has previously been associated to improvement in PMDD symptoms (Freeman et al., 2002) although different findings also are reported (Wang et al., 1996; Girdler et al., 2001).

The number of cycles in the study for Paper II is limited. We wanted to have cycles with luteal phase serum progesterone concentration > 15 nmol/L taken within the stipulated time frame (9 days preceding menses). The change in cycle length, most likely due to buserelin treatment, made it difficult to anticipate the optimal time for blood sampling. With a more frequent blood sampling, we could have used actual serum steroid levels instead of transformed z-score levels, although z-scores are representative to the specific day of the menstrual cycle that it is taken on.

However, carry over effects from GnRH agonist is unlikely in this study as only ovulatory cycles are included. Furthermore, none of the placebo cycles in this study were directly preceded by a cycle with GnRH agonist treatment.

The placebo effect in PMDD treatment is powerful, and this effect also treats other medical conditions – pain (Rowbotham, 2001), major depression (Walsh et al., 2002), acute migraine (de Craen et al., 2000), and Parkinson’s disease (de la Fuente-Fernandez et al., 2002). In a meta-analysis of controlled treatment trials for PMDD, the placebo response ranged from 6% to 35% (Yonkers et al., 1997d). The mechanism behind this phenomenon is not clear, but different possibilities have been suggested. A placebo-induced effect on the opioid system with release of endogenous endorphins (Kop et al., 1989) and modulation of transmitter systems like the dopaminergic system (Sher, 2003) has been suggested. It is possible that the placebo response in this study is mediated by endorphin release, with a decrease in gonadotropin secretion, resulting in decreasing progesterone and allopregnanolone levels with consequent improvement in symptom severity. Another possibility might be a direct influence of the endorphins on the symptoms with a decrease in symptom severity and a lowering of gonadotropins and allopregnanolone levels simultaneously.
The absolute level in allopregnanolone concentrations might not be the only explanation to the appearance of symptoms, but a combination of an altered GABA\textsubscript{A} receptor sensitivity and a possible tolerance development to these neuroactive agents (Zhu et al., 2004) may render certain women less sensitive to the effect of allopregnanolone in the luteal phase of the menstrual cycle. In postmenopausal women who receive sequential progesterone and estradiol as a hormone therapy, the symptom severity increases as the serum level of allopregnanolone increases to a certain level. After this, the severity evens out and eventually decreases displaying an inverted U-shaped relation between the symptom severity and the allopregnanolone concentration (Andreen et al., 2005; 2006). If this hypothesis is true, our subjects were on the lower end of the U-shaped curve as symptom improvement was accompanied by decreasing allopregnanolone levels.

**Effects of a Low Dose of Alcohol in Women with PMDD and Healthy Women**

The main finding was that PMDD patients had a decreased sensitivity to low doses of alcohol in the late luteal phase compared to the mid-follicular phase. Thus, changes in circulating ovarian steroids might indirectly influence alcohol response in this particular subgroup of women, whereas our and previous studies have failed to detect menstrual cycle effects in alcohol response among healthy women.

The decreased saccadic eye movement sensitivity in response to alcohol in the late luteal phase might contribute to previous findings of increased alcohol consumption in PMDD/PMS women in the late luteal phase, which is not seen in healthy women (Christensen et al., 1989; Charette et al., 1990; Mello et al., 1990; cf. Tobin et al., 1994). However, data regarding alcohol consumption among women with PMDD must be interpreted with caution. Many of these studies are hampered by methodological problems, such as retrospective reportings of premenstrual symptoms, insufficient definition of menstrual cycle phase, and insufficient confirmation of ovulatory cycles. It must be emphasized that we were unable to find signs of increased alcohol consumption in our PMDD patients. In fact, their report of alcohol use was lower than what the controls reported although there was no significant difference.

Apparently, PMDD patients are more susceptible than control subjects to the neuroendocrine changes induced by circulating levels of estradiol and progesterone during the menstrual cycle. Benzodiazepines, also acting via the GABA\textsubscript{A} receptor have been shown to affect PMDD patients and controls
differently. Using saccadic eye movement measurement, studies have shown that women with PMS have a reduced sensitivity to benzodiazepines and pregnanolone. The reduced responsiveness was more pronounced in the luteal phase compared to controls (Sundström et al., 1997a,b, 1998a). As alcohol also acts via the GABA_A receptor, our findings are in line with these previous studies, together pointing towards a reduced functional GABA_A receptor sensitivity in PMDD patients.

There was no change among control subjects in saccadic eye movement sensitivity in response to alcohol across the menstrual cycle. Although subjects were tested at a comparably low blood alcohol concentration, this finding is in line with previous studies on alcohol effects across hormonally distinct phases of the menstrual cycle (Holdstock and de Wit 2000; Mumenthaler et al., 2001a, b). Some studies have indicated a relationship between alcohol-induced performance impairment, mood deterioration, and the menstrual cycle (Jones and Jones, 1976a; Sutker et al., 1987a), but the majority of studies on alcohol-related cognitive, behavioral, and sedative effects have not demonstrated any significant influences of ovarian steroids and menstrual cycle in healthy women (Linnoila et al., 1980; Brick et al., 1986; Niaura et al 1987; Holdstock and de Wit, 2000; Mumenthaler et al., 2001a,b).

The initial effect of alcohol on the saccadic eye movements was in the present experiment recorded after 5 minutes of infusion. At this time, alcohol had resulted in a significant effect on both SEV and saccade deceleration in both groups and cycle phases. Since the infusion dose rate of alcohol was only 0.007 g/kg/min, these initial responses were induced by 0.033 g/kg alcohol. Thus, alcohol was able to influence saccade movements after a brief exposure to low concentrations. This finding is in line with previous studies demonstrating effects of very low doses of alcohol on electrooculographic variables, auditory-evoked potentials, and behavioral measures (Davidson et al., 1997; Cohen et al., 1998; Pearson and Timney, 1998; Arden and Wolf, 2000). Our results indicate a surprisingly high potency of alcohol, which could be due to a specific receptor action (Mihic, 1999). As stated in the introduction of this thesis, the GABA system could be a candidate for such a specific action. Another factor that can influence the brain response of an intravenous infusion of alcohol is at what rate the brain is exposed to the drug (Korkmaz and Wahlström, 1997).

There are a number of limitations to this study. Baseline alcohol consumption was based on retrospective reports of alcohol use during the four weeks preceding the study. Retrospective reports of alcohol consumption are less valid than prospective reports, and it can be assumed that the reported use of alcohol is underestimated. The assessment of alcohol consumption was not
aimed for evaluating drinking patterns among the study subjects, but for excluding subjects with abuse or heavy drinking. Based on the retrospective reports, it is difficult to draw any conclusions about the actual alcohol consumption in these two groups of healthy subjects other than the fact that the study subjects appeared to be light or moderate consumers. There was no record of alcohol consumption during the testing period, but subjects were instructed to not consume any alcohol within 24 hours of the tests.

Family history of alcohol abuse was not assessed. A reduced response to alcohol has been reported among individuals with a family history of alcoholism (Pollock, 1992; Schuckit et al., 2000; Evans and Levin, 2003), which could have contributed to our findings.

For the alcohol challenges, the luteal phase tests were scheduled according to the subjects’ previous records of menstrual cycle length rather than from luteinizing hormone assays. Cycle length variability is a problem in any study across the menstrual cycle in particular when experiments are to be planned in the late luteal phase. However, because regular menstrual cycles was one of the inclusion criteria for the study, we were able to schedule all subjects within the stipulated time-frames and no subjects had to be rescheduled because their menstrual bleeding started before the luteal phase testing were completed.

**The Effect of a Low Dose of Alcohol on Allopregnanolone Concentration**

In Paper IV, we were unable to replicate prior findings of increased allopregnanolone levels following alcohol infusion. The infusion of a low dose of alcohol employed in our study did not increase allopregnanolone levels; in fact, a small decrease in allopregnanolone levels in response to alcohol was noted in the late luteal phase. The alcohol doses used in previous animal studies has been substantially higher and route of administration has also differed. Prior studies in laboratory animals have used alcohol doses between 1.0 g/kg intraperitoneally (Barbaccia et al., 1999) and 1.3 - 4.0 g/kg intraperitoneally (VanDoren et al., 2000), whereas the current study employed intravenous alcohol doses of 0.2 g/kg.

Following the publication of our study, our findings have been confirmed by two studies. Holdstock and de Wit (2005) tested alcohol response in 12 healthy women in both the follicular and luteal phase together with nine men. Subjects consumed two alcoholic beverages (0.7 g/kg and 0.8 g/kg) and placebo. Alcohol had no effect on allopregnanolone plasma levels in either phase of the menstrual cycle neither in women nor had it any effect on allopregnanolone
plasma levels in men. Similar to our findings, alcohol did not increase cortisol in men or in women. A decrease in progesterone levels has also been observed after acute intake of 3 standard drinks of alcohol. This was seen together with decreased levels of allopregnanolone and a decrease in the ratio of pregnenolone to progesterone (Pierucci-Lagha et al., 2005), indicating an altered conversion of pregnenolone to progesterone. The same findings with a decrease in progesterone levels have been seen, especially in the luteal phase (Sarkola et al., 1999), although some researchers have not been able to reproduce these findings (Välimäki et al., 1983; Holdstock et al., 2005). Because we did not investigate other neurosteroid levels than allopregnanolone, we did not replicate the findings.

Decreasing allopregnanolone levels following alcohol consumption was in fact already reported in a small study from 1969. Allopregnanolone as well as pregnanolone levels decreased following ingestion of 0.5 g/kg alcohol (Cronholm et al., 1969). Their explanation to the decreasing allopregnanolone levels was that it was due to a change in the oxidation-reduction state of keto/hydroxysteroids, which in turn was due to a decrease in the NAD⁺:NADH ratio during alcohol oxidation (Cronholm et al., 1968; 1969; 1970; Axelson et al., 1981). When alcohol is oxidized by the enzyme alcoholdehydrogenase, NAD⁺ is reduced to NADH to produce acetaldehyde, and the alcohol-induced change in redox potential would suggest decreasing levels of allopregnanolone in favor of its metabolite allopregnanediol. Similarly, testosterone levels and estradiol levels (17-hydroxysteroids) increase in premenopausal women following alcohol ingestion as a result of an increased reduction from androstenedione and estrone, respectively (Sarkola et al., 2000; 2001b). Cronholm suggested that this way of steroid metabolism might also include the ratio between cortisol and cortisone and between pregnenolone and progesterone (Cronholm et al., 1969). When men were given 4-methylpyrazole (an inhibitor of alcohol metabolism), no effect of alcohol on testosterone or androstenedione levels were seen (Sarkola and Eriksson, 2003).

The study findings might indicate that the anxiolytic response of alcohol is not mediated by allopregnanolone, at least not with low doses of alcohol. However, the study design does not allow us to draw any firm conclusions on the anxiolytic response because we did not use any measure for anxiety levels during the alcohol and placebo infusions.
Alcohol and HPA-Axis Function

The present study did not indicate any alcohol-induced changes in HPA-axis activation, which might be due to study design and/or dose of alcohol. It is known that the HPA system can be activated by both alcohol and stress. Humans have a circadian rhythm of cortisol secretion, with the lowest point in the early morning and the peak in the late morning hours. Test sessions in the present study were not standardized with respect to time of day, because measurement of cortisol levels was a secondary aim of the study and cortisol levels were used for assessing the stress response in relation to the placebo infusions. The absence of alcohol-induced increase in cortisol levels could be due to the low alcohol dose leading to a stress reduction. It has been demonstrated that acute alcohol intoxication produces an increase in cortisol levels in both men and women (Frias et al., 2002) and an increased corticosterone secretion in female rats (Ogilvie and Rivier, 1997). However, moderate doses of oral alcohol (1.0 g/kg) in nondrinkers result in a slight decrease in plasma cortisol concentration (Aguirre et al., 1995; Inder et al., 1994), or no changes in cortisol response in humans (Gianoulakis et al., 1996; Holdstock and de Wit, 2005). In the present study, cortisol levels decreased during both placebo and alcohol infusions, most likely due to a reduction in stress response throughout the test sessions, but possibly also due to diurnal changes.

Alcohol Response in Men and Women

The main finding in Paper V was the absence of gender-related differences in the saccadic eye movement sensitivity to low doses of alcohol.

Although the study findings were negative, the major strength of our study was that we were able to obtain similar blood alcohol concentrations in female and male subjects. By using an intravenous route of administration, good control of blood alcohol concentration was achieved, as indicated by the absent gender difference in blood alcohol concentrations after 25 minutes of alcohol infusion. In addition, possible gender related differences in absorption and first pass in metabolism was avoided. Gender differences in blood alcohol concentrations have been reported after oral intake of alcohol, whereas intravenous administration results in similar blood alcohol levels in men and women (Arthur et al., 1984; Goist and Sutker, 1985; Frezza et al., 1990).

Our findings agree with Blekher and colleagues (2002) who reported a decreased saccadic eye velocity after ingestion of alcohol in African-American
and non-Hispanic white college students. No gender differences were observed in their study in any of the studied saccade measurements (Blekher et al., 2002). We chose to compare healthy women without PMDD with men since we wanted to have a group of women that were not subjectively affected by their menstrual cycle. Males consumed significantly more alcohol than females (p < 0.05), but according to the reported weekly consumption both groups were light to moderate consumers. This difference in alcohol consumption might reflect the fact that males did not report any feeling of intoxication until after 15 minutes of alcohol infusion, whereas females started to feel intoxicated five minutes after the start of the infusion. A possible explanation can be a tolerance/learning effect due to the overall higher alcohol consumption among males. Males also rated themselves less intoxicated throughout the test session although this finding was not statistically significant. There was a positive correlation between sedation scores and reported amount of alcohol use, and this finding strengthens the fact that the study subjects were not heavy alcohol consumers. A higher consumption would have resulted in a decreased response to the effect of alcohol.

It is possible that with a higher dose of alcohol we would have also been able to demonstrate a gender difference in the saccadic eye movement measurements. Using a divided attention test with a fairly high dose of alcohol (0.76g/kg), gender related differences were evident, whereas at a lower dose (0.37g/kg) no difference between male and female subjects was found (Mills and Bisgrove, 1983).

Methodological Considerations

By merely reading the papers of this thesis, it might appear as though we have used different criteria for PMDD diagnoses or, even worse, studied different disorders. This is, however, not the case. As stated earlier, similar diagnostic procedures have been employed for all studies although we have been more or less successful in convincing our reviewers that we actually were studying PMDD patients. Also, at the time of the first study, the term premenstrual syndrome was still used by our research group, although DSM-IV criteria were applied. Reasons why PMDD diagnosis has been questioned are given below.

A limitation that was not evident when this study was conducted, but with time has become more and more evident, is the subscale used for measuring impact on daily life in patients with PMDD. Each step on the rating scale for impact on daily life has a specific explanation of how much the premenstrual
symptoms affect the daily life of women. The women were instructed to mark the highest item experienced during a specific day, regardless of whether they had experienced other items within the scale, with a lower grade. Many women have misinterpreted the scale, assuming that they had to experience all the impact variables below the one they actually experienced, which in turn may have made them choose a lower than possible rating for the daily impact. Furthermore, our scale for impact of daily life differs from other validated PMDD scales, which has been confusing for reviewers. If a woman indicates that her family notices her premenstrual symptoms, she then uses the scale step 2 out of maximum of 8. Without knowledge of the patient, it thus might appear that the severity of her symptoms is not sufficient to fulfill criteria for PMDD. At the same time, it is very rare that PMDD patients admit that their premenstrual symptoms affect their working capabilities; this may explain why the scale steps 6-8 are rarely used. However, all our patients have been recruited among patients who actively sought help for premenstrual symptoms at the department of Obstetrics and Gynecology (as opposed to newspaper advertisement). Because the patients contacted a gynecologist, we believe their symptoms are of a sufficient degree to fulfill severity criteria for PMDD.

Another limitation was that no standardized diagnostic instrument for evaluation of ongoing or past psychiatric history as well as history of alcohol and/or drug abuse and/or dependence was used to exclude subjects with ongoing and prior history of these disorders. PMDD diagnosis requires the exclusion of ongoing depressive or anxiety disorders, and in this study the exclusion of subjects with other psychiatric disorders was merely based on a semi-structured clinical interview and the absence of symptoms in the follicular phase according to the symptom rating scales. This is also one of the reasons why certain reviewers have questioned the PMDD diagnosis in our studies. However, based on extensive clinical experience in both academic studies and clinical trials, with or without structured psychiatric interviews, we are convinced that absence of follicular phase symptoms on daily ratings is equivalent to absence of underlying psychiatric disorder. This has also been validated in prior studies from our group (Hammarbäck and Bäckström, 1989b).

Future Investigations

We have used a low dose of alcohol in these studies. It would be interesting to use a higher dose of alcohol and compare the response in healthy men, healthy females and PMDD patients. It may be useful to look at the same measurements on study subjects who have never used alcohol and to look at
their response to alcohol. Much research on alcohol has focused on the genetic influence since the prevalence of alcoholism is up to four times higher among first-degree relatives of alcoholics. The suggested connection between earlier stress events in life like physical and sexual abuse, anxiety, and mood disorders, the interaction of hormones and neuroactive steroids, and drug abuse is an interesting and important field of research.
1. GnRH-agonist (buserelin) in a low dose effectively treats premenstrual symptoms like depression and irritability. In addition, feelings of cheerfulness and friendliness, and physical symptoms like swelling, and headache are improved.

2. There is a relation between improvement in symptom severity and decreasing levels of allopregnanolone. This relationship was evident both in subjects responded to low dose GnRH-agonist (buserelin) and within those who responded to placebo treatment.

3. Women with PMDD have a decreased sensitivity to a low dose of alcohol in the luteal phase compared to the follicular phase as measured by saccadic eye velocity and saccade deceleration. There was no menstrual cycle effect on any measured saccadic parameter in response to low dose of alcohol among healthy female controls. The sensitivity to alcohol does not differ between PMDD patients and control subjects.

4. There is no difference in the alcohol-induced effect on allopregnanolone levels between women with PMDD and control subjects. Low-dose alcohol reduces allopregnanolone levels, but only in the luteal phase.

5. Subjective feelings of intoxication and sedation increase after the low dose of alcohol in both males and females. Alcohol decreases SEV and increases saccade deceleration and saccade accuracy. There is no difference between males and females in any of the measured parameters.

These findings further strengthen the theory that women with PMDD have an altered GABA<sub>A</sub> receptor sensitivity especially in the luteal phase and that there seems to be a relation between serum allopregnanolone levels and negative mood severity.
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REFERENCES


Ben-Porath DD, Taylor SP. The effects of diazepam (valium) and aggressive disposition on human aggression: an experimental investigation. Addict Behav 2002; 27:167-177.


Blume SB. Women and alcohol: A review. JAMA 1986; 256:1467-1470.
Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors. Mol Pharmacol 2003; 63:53-64.


Davies PA, Hanna MC, Hales TG, Kirkness EF. Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit. Nature 1997; 385:820-823.


Eriksson E, Sundblad C, Lisjö P, Modigh K, Andersch B. Serum levels of androgens are higher in women with premenstrual irritability and dysphoria than in controls. Psychoneuroendocrinology 1992; 17:195-204.


Freeman EW, Rickels K, Sondheimer SJ, Polansky M, Xiao S. Continuous or intermittent dosing with sertraline for patients with severe premenstrual syndrome or premenstrual dysphoric disorder. Am J Psychiatry 2004; 161:343-351.


Golding JM, Taylor DL. Sexual assault history and premenstrual distress in two general population samples. J Womens Health 1996; 5:143-152.


Griffin ML, Mello NK, Mendelson JH, Lex BW. Alcohol use across the menstrual cycle among marihuana users. Alcohol 1987; 4:457-462.

Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of GABA_A receptors in the acute and chronic effects of ethanol. Psychopharmacology 1998; 139:2-19.


Luisi S, Petraglia F, Benedetto C, Nappi RE, Bernardi F, Fadalti M, Reis FM, Luisi M, Genazzani AR. Serum allopregnanolone levels in pregnant women: changes during


**Miczek KA**, Fish EW, De Bold JF. Neurosteroids, GABAA receptors, and escalated aggressive behavior. Horm Behav 2003; 44:242-257.


Sundström I, Nyberg S, Bäckström T. Patients with premenstrual syndrome have a reduced sensitivity to midazolam compared to control subjects. Neuropsychopharmacology 1997a; 17:370-381.


Sundström I, Andersson A, Nyberg S, Ashbrook D, Purdy RH, Bäckström T. Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. Neuroendocrinology 1998a; 67:126-138.

Sundström I, Bäckström T. Patients with premenstrual syndrome have decreased saccadic eye velocity compared to control subjects. Biol Psychiatry 1998b; 44:755-764.


Tabakoff B, Kiianmaa K. Does tolerance develop to the activating, as well as the depressant, effects of ethanol? Pharmacol Biochem Behav 1982; 17:1073-1076.


Torres JM, Ortega E. Alcohol intoxication increases allopregnanolone levels in female adolescent humans. Neuropsychopharmacology 2003; 28:1207-1209.


Uzunov DP, Cooper TB, Costa E, Guidotti A. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc Natl Acad Sci USA 1996; 93:12599-12604.


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Start datum: 5152 3
Skattnings dagens datum

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Födelsedatum:........................................................

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Start datum för skattningar:.............

Årtal:.............

Nedstämd

Bröstspännings

"Symptom-diagnoser" av Professor Torbjörn Bäckström
Skattningsskala nummer 3.

Förklaring till Påverkan

0=Ingen Påverkan
1=Jag märker
2=Familjen märker
3=Stör relationer i familjen
4=Undvikar socialt umgänge
5=Avstår från socialt umgänge
6=Svårigheter att arbeta
7=Ej klarat arbetet
8=Frånvaro från arbetet

Menstruationsblödning

Påverkan