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Review

Pre-menstrual steroids

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Abstract. A number of steroid hormones and their metabolites fluctuate in the circulation across the human menstrual cycle. In addition to their classic actions on the hypothalamo-pituitary-gonadal axis, many of these hormones act as 'neuroactive steroids' to alter the function of neurotransmitters, such as GABA, within central nervous system circuits. Clinically, these steroids are important because they have not only acute but also long-term effects, and 'withdrawal' properties. This review discusses the effects of steroids such as 3α-OH-5α-pregnan-20-one (3α,5α-THP or allopregnanolone) which alter GABA function in distinct ways dependent upon the time course of exposure, to either enhance or decrease inhibition in the brain. These effects are discussed in light of recent clinical findings which seek to further characterize the steroid milieu which underlies pre-menstrual dysphoria.

Key words. Allopregnanolone; withdrawal; α4 subunit; $GABA_A$ receptor; pre-menstrual syndrome; PMS; premenstrual dysphoric disorder; PMDD; hippocampus; rat.

Introduction

The study of neuroactive steroids is a relatively new phenomenon – both in terms of steroid-neurotransmitter interactions and the psychotropic actions of these agents. In addition to the well-established effects of these steroids on classic intracellular receptors [1], certain steroids, a metabolite of progesterone, 3α-OH-5α-pregnan-20-one (3α5α-THP or allopregnanolone) [2], and a sulfated precursor of testosterone DHEAS, [3] have neuromodulatory effects. Both steroids exert effects on traditional neurotransmitter receptor systems within circuits in the brain which may result in altered affect. One physiologically relevant end-point of these steroid effects is pre-menstrual syndrome (PMS). An estimated 70% of all women experience some type of dysphoria across the menstrual cycle, typically during the late luteal phase, a time of declining hormone levels [4]. Those afflicted more seriously, 5–10% of the population, experience adverse symptomatology shortly after the mid-cycle peak in circulating steroid levels, a condition termed 'premenstrual dysphoric disorder' (PMDD). Both syndromes are linked to hormonal events, i.e., hormone withdrawal vs hormone exposure, but clearly stem from different etiologies. A clearer understanding of neuroactive steroid effects in the brain may then lead to a clearer understanding of the potential psychotropic and cognitive effects which accompany fluctuations in these endogenous steroids under normal and pathophysiological conditions.

Psychoactive and sensorimotor effects of menstrual homones

The human menstrual cycle is a 28 ± 7-day event which begins at the onset of menses (see fig. 1), and includes an estrogen-dominant follicular phase of variable duration which culminates in a mid-cycle peak of 17β-estradiol (E_2) and ovulation, followed by a 12- to 14-day progesterone-dominant luteal phase. In addition to E_2 and prog-

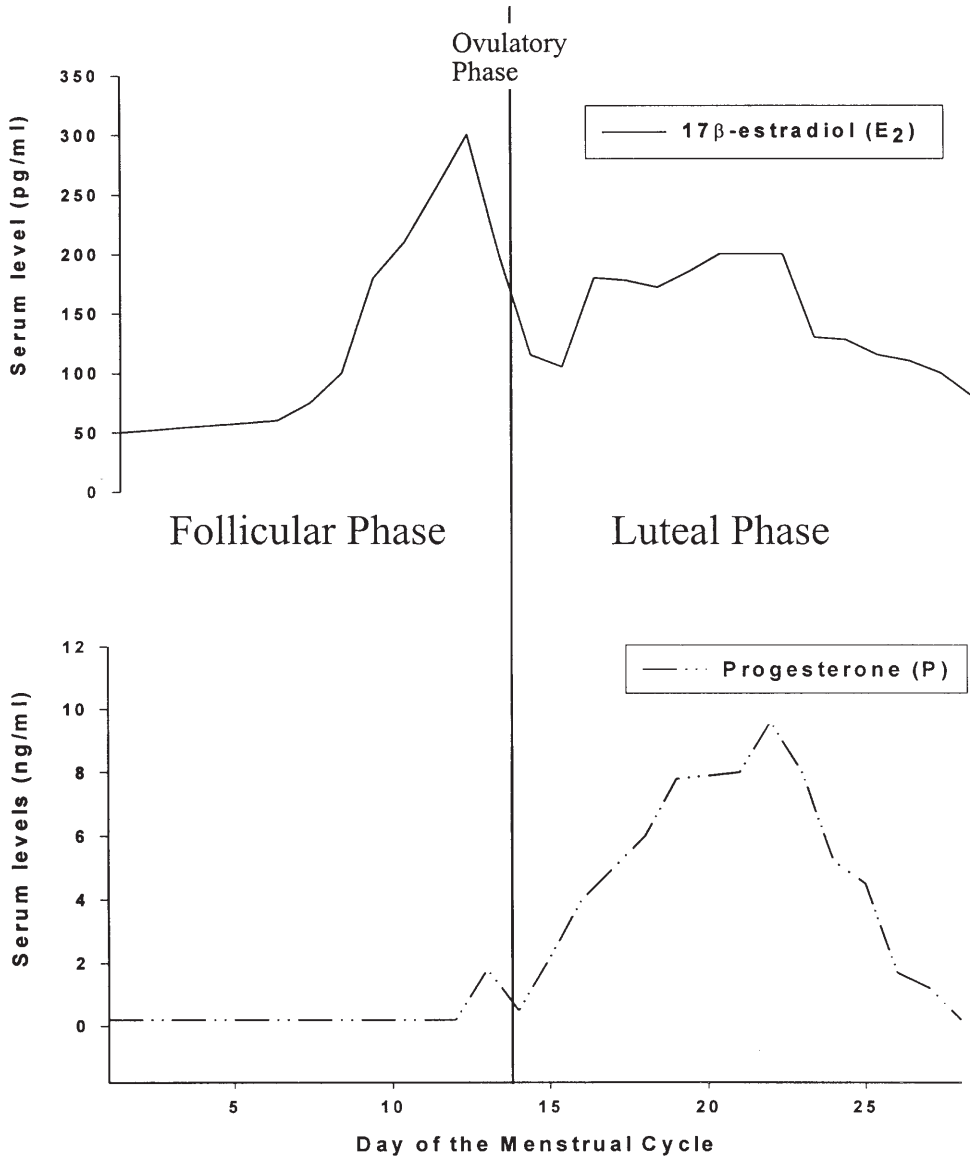

Figure 1. The 28 day menstrual cycle. Elevations in 17β-estradiol (E_2) peak during the follicular phase, preceding ovulation, while progesterone levels are increased in the luteal phase. Both hormone levels decline before the onset of menses (day 1 of the next cycle). Fluctuations in associated metabolites of these hormones are described in the text.

esterone, a variety of metabolites, such as 3α,5α-THP and 17β-diol, are also increased, as are androgens such as dehydroepiandrosterone (DHEA), its sulfated form, DHEAS, and testosterone [5–7].

Over the years, the clinical literature has suggested that estrogen, either fluctuating endogenously or administered exogenously, is associated with activating effects on the sensorimotor system and affect in susceptible individuals [8]. Included in this array of end-points are euphoria, anxiety, increased arousal and improved attentional mechanisms, improved verbal memory [9, 10], facilitation of rhythmic movement [8, 11], including improved performance on the peg-and-board task [12], improved balance and coordination, and a lowered sensory threshold for a variety of modalities [8]. In contrast, progesterone, associated with the luteal phase or administered exogenously, has characteristically been associated with depressive end-points [13], as well as increased feelings of tranquility [14] and improved performance on tasks requiring a response delay [8]. These concepts have recently been confirmed in a recent study assessing cortical excitability across the phases of the menstrual cycle [15].

The late luteal phase, a time of declining hormone levels, is a period most often associated with the dysphoria which is often labeled PMS [4]. PMS is a term encompassing diverse symptomatology which includes both somatic and affective disturbances. A smaller percentage of women also experience dysporia shortly after the mid-

cycle peak in ovarian hormones, a more debilitating syndrome which the DSM-IV (American Psychiatric Association) has classified as PMDD [4]. These cyclic disturbances in mood have been grouped into three categories, which include the following symptoms: (i) anxiety, irritability, internal tension and emotional lability, (ii) depression, anhedonia, sleep disturbances, and (iii) aggression. The complexity of the symptomatology probably results from multiple mediating factors. That a release from inhibitory influences is the hallmark of the late luteal phase is suggested by the increase in seizure susceptibility reported at this time in individuals with preexisting epileptic foci (i.e., 'catamenial epilepsy') [16, 17], a phenomenon also observed during the mid-cycle peak in E_2. Increases in seizure activity do not occur during the hormone withdrawal phase, but at a time when progesterone levels are at their nadir, suggesting a higher threshold for seizure induction compared with reports of adverse mood.

Steroid pharmacology

Estradiol

E_2 is an ovarian steroid hormone which predominates during the follicular phase but reaches a second smaller peak during the luteal phase. Its effects on the central nervous system (CNS) are only beginning to be understood. Early work in this area demonstrated that, at physiological concentrations, iontophoretically applied succinate-conjugated steroid altered neuronal activity in the basal hypothalamus [18]; in some cases, excitatory effects were seen which may be mediated through attenuation of $GABA_B$ receptor activity [19]. In addition, in vivo administration of the steroid can increase neuronal responses to glutamate, applied iontophoretically to cerebellar Purkinje cells [20]. This effect was relatively rapid, with an onset between 20 min–1 h, and, unlike conventional effects of this steroid on reproductive behavior and luteinizing hormone-releasing hormone (LHRH) release, was not blocked by protein synthesis inhibitors or the classic anti-estrogen anisomycin [20]. It was also replicated by direct, local application of the steroid [21]. Although recent studies suggest that a novel form of the E_2 receptor, ER-β, is localized to Purkinje cells [22], the involvement of this receptor in the neuromodulatory actions of E_2 is not yet resolved. Later studies revealed that these effects were directed toward both the Quis/AMPA receptor as well as the N-methyl-D-aspartate (NMDA) receptor subtype of the glutamate receptor [23]. More relevant for the potential psychotropic and cognitive actions of the steroid, similar effects of E_2 on glutamate and NMDA-evoked responses in hippocampal slices have been reported by Moss and colleagues. In this case, acutely applied E_2 potentiated excitatory responses mediated by kainate-specific receptors via a G-protein mechanism [24, 25], and after E_2 priming, potentiated excitatory synaptic input [26]. These effects may be specific for the NMDA receptor, as increases in MK-801 binding occur following E_2 treatment; specific increases in the NR1 subunit of this receptor complex have also been reported [27]. Second, increases in dendritic spine density are seen after in vivo treatment with E_2 or during the E_2-dominant phase of the estrous cycle, an effect resulting in increases in the input:output relationship of hippocampal pyramidal neurons, suggesting the potential for amplification of relevant input [28, 29]. Other novel effects of this steroid include increased conductance of L-type calcium channels after acute administration [30]. The conclusion from these studies is that, at least based on pharmacological data, E_2 exerts potentiating effects on excitatory synaptic input.

Progesterone and metabolites

The C21 steroid progesterone is formed by the corpus luteum of the ovary, where it is released into the circulation during the luteal phase to act predominantly on cytosolic progesterone receptors with ensuing genomic actions [31]. The adrenal is another source of this steroid, where release can be a response to stressful stimuli. Progesterone can also be formed in the brain, de novo, from cholesterol via a side chain cleavage enzyme (cytochrome P-450), and thus can also be classified as a neurosteroid [31, 32]. Early studies from this laboratory demonstrated that in vivo administration of progesterone rapidly potentiates neuronal responses to GABA while decreasing glutamate responses [33], both iontophoretically applied to cerebellar Purkinje cells. The latter effect may be mediated via the sigma-1 receptor [34]. The former effect was specific for the $GABA_A$ receptor (GABAR) subtype [35,35], and was not due to actions on conventional progesterone receptors but was in fact mediated by a metabolite, 3α,5α-THP a potent GABA-modulatory agent (see fig. 2). This was shown by studies which demonstrated that the GABA-modulatory effect of progesterone was prevented by drugs such as the 5α-reductase blocker 4MA (17β-N, *N*-diethylcarbamoyl-4-aza-5α-androstan-3-one) which blocks conversion of

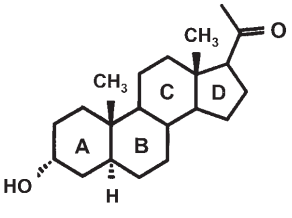

Figure 2. Structure of one of the most potent neuroactive steroids, 3α,5α-THP (3α-OH-5α-pregnan-20-one or allopregnanolone).

this steroid to its metabolites [36]. Progesterone is converted in the periphery or the CNS via 5α-reductase to 5α-DHP, and further converted by 3α-hydroxysteroid dehydrogenase (3α-HSD) to 3α,5α-THP [31]. Additionally, potentiation of GABAergic inhibition was also observed after direct local application of 3α,5α-THP [37]. Thus, these initial studies suggest that elevations in circulating levels of progesterone can enhance GABAergic inhibition via local conversion to 3α,5α-THP within the CNS.

3α-OH-5α-pregnan-20-one

3α,5α-THP was first shown to potentiate GABA-gated current (see fig. 3) in spinal cord and hippocampal neurons using whole-cell patch clamp techniques [2, 38]. Further evaluation revealed that 3α,5α-THP prolonged single-channel openings of the GABA-gated Cl^- channel in a manner similar to barbiturates at physiological (nM) concentrations [39] without altering channel conductance state, and at higher doses had a GABA-mimetic effect [38]. This effect exhibited marked stereospecificity, as the 3β-OH isomer is without effect [2], while the 5β-isomer is almost as potent [40]. The latter hormone is also an endogenous hormone in the human. Other steroids, such as progesterone itself, testosterone, and E_2 are all without effect at physiological concentrations [41, 42]. In semi-intact hippocampal circuits, 3α,5α-THP prolongs the decay time of GABAergic synaptic current [43], thus enhancing inhibition.

The $GABA_A$ receptor

The GABAR has a pentameric structure (see fig. 4) comprised of varying combinations of α (1–6), β (1–4), γ (1–3), δ, ε, π, and ρ (1–3) subunits [44–47]. As such, it mediates most fast inhibitory synaptic current in the adult CNS. Each subunit is further comprised of four membrane-spanning α helices; the M2 segments border the central Cl^- channel. The most common GABAR isoform is 2α,2β, 1γ [48], although other combinations exist. A variety of drugs, including benzodiazepines, barbiturates, anesthetics and, to some extent, alcohol bind to the GABAR at unique sites and act to potentiate GABA-gated current, as does 3α,5α-THP. A number of studies have used transient expression systems to test whether variations in GABAR isoform alter steroid sensitivity. Although varying the α and β subtype produces some change in steroid sensitivity [40, 49, 50], recent evidence suggests that novel GABAR subunits may confer steroid sensitivity or insensitivity to the receptor, In particular, the δ subunit produces increased steroid sensitivity [51], as transgenic mice which are missing this subunit are relatively steroid insensitive, assessed using sleep time as a measure of GABA function. Furthermore, another recent study [52] has established that addition of the ε subunit confers steroid insensitivity, along with insensitivity to anesthetics, barbiturates, and benzodiazepines.

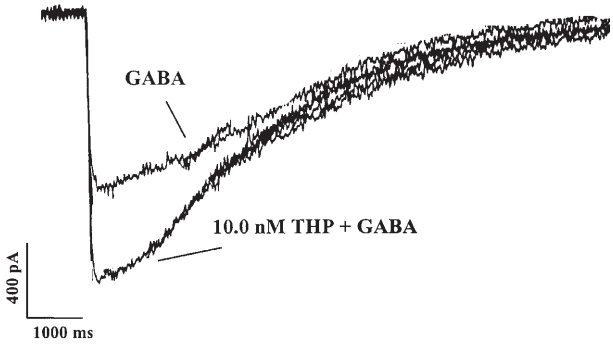

Figure 3. 3α,5α-THP potentiation of GABA-gated current. The concentration-response effect of 3α,5α-THP (THP) is illustrated here as assessed using whole-cell patch clamp procedures on pyramidal neurons acutely isolated from the CA1 hippocampus of an adult female rat in diestrus. Concentrations of the steroid as low as 10.0 nM significantly potentiate GABA-gated current (representative of effects recorded from 14 of 17 cells).

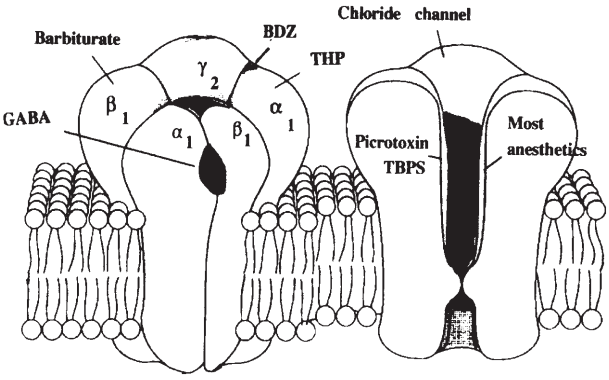

Figure 4. Theoretical binding site for neurosteroids on the $GABA_A$ receptor. The $GABA_A$ receptor is a pentameric structure; each of its subunits is in turn comprised of four membrane-spanning α helices. The TM2 region surrounds the ligand-gated Cl^- channel. This receptor contains distinct binding sites for GABA agonists, as well as different classes of GABA modulators. GABA agonists are thought to bind in the cleft between the α and β subunits, while benzodiazepines bind between α and γ subunits. Anesthetics and ethanol bind to the TM2 region. Binding domains for barbiturates are thought to be dependent upon the β subunit, while the binding site for the GABA-modulatory steroids such as 3α,5α-THP (THP) has not yet been identified.

Behavioral effects of 3α,5α-THP

In a manner similar to other GABA-modulatory drugs, 3α,5α-THP is also anxiolytic [53, 54], anti-convulsant [55], sedative [56] and at high doses can act as a general anesthetic [57]. Studies by Bitran and colleagues [53, 58]

were the first to establish that this steroid or the parent compound progesterone administered systemically or intraventricularly decreased anxiety, assessed by open arm entries using the elevated plus maze. Furthermore, blockade of progesterone conversion prevented the anxiolytic effects of progesterone in this paradigm [59]. More recent results from this group have established that direct administration of *3α,5α*-THP into the dorsal hippocampus produces a rapid anxiolytic effect [60]. Direct administration of 3α,5α-THP into the amygdala [54], another area which may be more important for generating acute anxiety, has also been shown to reduce anxiety, further establishing this compound as an anxiolytic agent, comparable to benzodiazepines and barbiturates. Other GABA-modulatory steroids are also anxiolytic. In particular, the reduced metabolite of 3α,5α-THP, 5α-pregnan-3α,20α-diol, is a pregnanediol and a GABA-modulatory steroid which increases GABA-gated current in transient expression systems, although with a reduced efficacy compared to 3α,5α-THP [40]. It is also anxiolytic at doses much lower than observed to produce motor impairment. Fluctuations of this hormone would also parallel those of progesterone and 3α,5α-THP across the luteal phase of the menstrual cycle, which may then contribute to alterations in mood.

Hormone withdrawal

One characteristic of GABA-modulatory drugs, such as the benzodiazepines, barbiturates, and alcohol, is that after chronic exposure, they result in tolerance [44], and after abrupt discontinuation, they produce withdrawal sequelae, producing symptoms such as anxiogenesis, irritability and, in extreme cases, seizure induction [61]. Our laboratory investigated whether 3α,5α-THP, as a GABA-modulator, could also exhibit tolerance and withdrawal properties under similar conditions. Initially, we established that chronic administration of progesterone for 5 days resulted in anxiogenic withdrawal properties, 24 h after discontinuation of steroid administration, assessed using the defensive burying paradigm and light:dark transition [62]. Following progesterone withdrawal, there was a 70% increase in the duration of and an 80% decrease in the latency to burying using the defensive burying paradigm, compared to controls, an effect consistent with an anxiogenic effect. This anxiogenic effect of hormone withdrawal was prevented by prior administration of indomethacin, a compound which prevents conversion of progesterone to its GABA-modulatory metabolite 3α,5α-THP, and could be mimicked by administering 3α,5α-THP directly [62], and then assessing withdrawal effects after abrupt cessation of its administration. These findings suggest that, in fact, it is abrupt discontinuation of 3α,5α-THP exposure following administration of its parent compound, progesterone, which produces withdrawal-associated increases in anxiety. An anxiogenic response to hormone withdrawal was further demonstrated using pseudopregnancy induction [63], which results in endogenous ovarian production of progesterone leading to elevations in circulating and CNS levels of 3α,5α-THP. Following ovariectomy or administration of a 5α-reductase blocker, finasteride, to directly precipitate 3α,5α-THP withdrawal, significant increases in anxiety were also observed [63].

In addition, progesterone withdrawal results in an increase in seizure susceptibility [64, 65] assessed by evaluating seizure severity following induction with picrotoxin or a β-carboline benzodiazepine inverse agonist. In this case, seizure activity was quantified using a score which consolidated the frequency, latency, and duration of seizure-like activity with seizure severity. This paradigm produced fivefold increases in seizure score following progesterone withdrawal compared to only minimal convulsant effects of these compounds observed under control conditions. Studies conducted by Frye and Bayon [66] have also shown increased seizure susceptibility after progesterone withdrawal, assessed using kainate-triggered and perforant pathway-induced seizures. Increased seizure susceptibility is known to occur following withdrawal of several GABA-modulatory drugs, such as alcohol, benzodiazepines, and barbiturates, which can, in the case of alcohol, produce persistent convulsant states where seizure activity is generated de novo [61].

The withdrawal effects of progesterone were shown to be due to upregulation of the α4 GABAR subunit [64]. Withdrawal from progesterone following a 3-week multiple-withdrawal paradigm resulted in a sixfold increase in α4 subunit protein (see fig. 5), accompanied by threefold increases in mRNA for the subunit. This subunit has been characterized as benzodiazepine insensitive [67], with a distinctive pharmacological profile which includes atypical responses to traditional GABA-modulatory drugs [68]. Following progesterone withdrawal, the pharmacology of the GABA-gated current was consistent with that reported for α4-containing GABAR. (In all cases, the GABA-gated current was recorded from acutely dissociated pyramidal cells using whole-cell patch clamp techniques.) The most striking of these changes was a benzodiazepine insensitivity (see fig. 5). In addition, there were atypical responses to two benzodiazepine inverse agonists, RO15-4513 and the β-carboline βCCM [64]. In contrast to the attenuation of the benzodiazepine response, there was an increase in the GABA-modulatory effect of applied barbiturates [64]. Suppression of the expression of the α4 subunit using antisense technology prevented the pharmacological changes observed following progesterone withdrawal, as well as the increase in seizure susceptibility also ob-

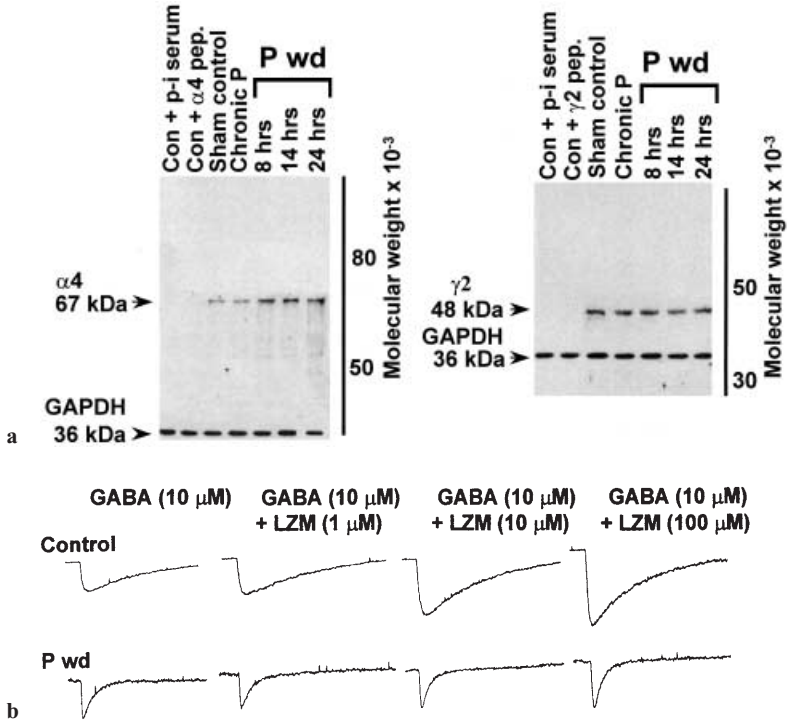

Figure 5. Withdrawal effects of progesterone. (*a*) Progesterone withdrawal increases $GABA_A$ receptor α4 subunit levels. Con, control; pep., peptide; p-i, pre-immune. Western blot analysis of α4 (67 kDa) (left) and γ2 (48 kDa) (right) subunits with a GAPDH (36 kDa) control protein. (*b*) GABA-gated currents from the CA1 hippocampus of control animals or after P withdrawal (P wd) reveal a marked insensitivity to the GABA-modulatory benzodiazepine lorazepam (LZM) across a range of concentrations following P wd, an effect consistent with the observed upregulation of the α4 GABAR subunit [reprinted with permission from ref. 64].

served at this time, thus suggesting a role for this subunit in mediating these changes in pharmacology at the whole-cell level.

In addition to altering GABA pharmacology, progesterone withdrawal also resulted in a marked sixfold decrease in the decay time for the GABA-gated current [63–65], as observed under both semi-equilibrium and non-equilibrium conditions. In contrast, no change in peak GABA-gated current or the GABA EC_{50} was observed. This decrease in decay time would decrease total charge transfer and decrease total GABA inhibition, an effect consistent with the increase in seizure susceptibility and increased anxiety observed following progesterone withdrawal.

The relevance of these findings for PMS may be specific for the subtype of dysphoria associated with the decline in progesterone and 3α,5α-THP in the week preceding menses. Benzodiazepine insensitivity has been reported in women with PMS [69], consistent with this model, but the increase in barbiturate sensitivity may have implications for the sedative potential of these compounds during the late luteal phase in PMS sufferers.

Other effects of hormone withdrawal

Other laboratories have studied the effect of hormone withdrawal on the GABA system. In one recent study, GABAR subunit composition in rat hippocampus and cortex was evaluated during pregnancy and the post-partum period [70], a time of progesterone withdrawal. The α4 subunit, in contrast to other subunits tested, increased significantly during the post-partum period in the hippocampus, an effect prevented by prior administration of finasteride, which prevents conversion of progesterone to its GABA-modulatory metabolite [70]. These results also suggest that a precipitous decline in CNS levels of 3α,5α-THP produces increases in hippocampal levels of the GABAR α4 subunit, results consistent with those from this laboratory.

In magnocellular oxytocin neurons of the hypothalamus [71, 72], the post-partum period is associated with a decrease in the α1:α2 subunit mRNA ratio, which is also well-correlated with an increase in the decay time constant of GABAR-mediated inhibitory post-synaptic currents. That the increase in α2 levels mediates the physiological change was suggested by the finding that antisense deletion of the α2 subunit prevented this change. More recent findings from this group suggested that α4 levels are also increased in these neurons during the post-partum 'progesterone withdrawal' period. However, insensitivity to the

GABA-modulatory effects of 3α,5α-THP also develops at this time, leading to an overall acceleration in the decay time, and less inhibition during the time of parturition when neuronal activity in this area would be required for increasing uterine contractility.

Neurosteroid exposure

In addition to withdrawal, shorter-term exposure to steroids can result in altered GABA pharmacology, in many cases as a result of alterations in GABAR subunit composition. Several in vitro studies have demonstrated that after 48 h exposure to steroids, cultured hippocampal and cortical neurons exhibit decreased sensitivity to the GABA-modulatory effects of 3α,5α-THP, as well as cross-tolerance to other GABA-modulatory compounds such as benzodiazepines [72–76]. As a corollary to this, chronic exposure to benzodiazepines also reduces the GABA-modulatory actions of 3α,5α-THP, suggesting that cross-tolerance may be something of a universal phenomenon for GABA-modulatory drugs. In addition, short-term administration of either P or 3α,5α-THP suppresses mRNA levels for the α1 and, to some extent, α2 subunit in both hippocampus and dentate gyrus, following E_2 pre-treatment [72, 77]. In contrast, P increases the γ2 subunit in the hippocampus, but produces no change in any of the β subunits. E_2 alone has no effect on steroid-induced changes in GABAR subunit composition, but exerts a permissive effect on the ability of P to alter GABAR subunits [77].

Recent work from this laboratory has demonstrated that short-term in vivo exposure (48–72 h) to either progesterone or 3α,5α-THP increases the α4 GABAR subunit as assessed both by Western blot analysis of the protein levels and semi-quantitative RT-PCR analysis of mRNA levels [78]. Under conditions of continuous hormone exposure, α4 levels decrease to control levels by 4–5 days. This increase in α4, as previously demonstrated under conditions of hormone withdrawal, was associated with an increase in anxiogenic behavior, assessed using the elevated plus maze. One possible mechanism for the increase in anxiety is a decrease in GABAergic inhibition which would be produced by the decrease in decay time constant observed. In summary, both short-term (2–3 day) exposure to the GABA-modulatory steroid 3α,5α-THP, as well as withdrawal from this steroid, result in upregulation of the α4 GABAR subunit. Both increases are transient events which are linked to decreases in GABAergic inhibition and a near total benzodiazepine insensitivity.

As discussed below, in PMDD, adverse symptomatology can also be observed within several days after the mid-cycle peak in ovarian hormones [4], thus suggesting a clinical relevance for the present observations. However, these data may only partially explain a possible etiology for the subtype of PMS triggered by the mid-cycle peak in hormones, which does not appear to be as transient an event as described by the present data. The possible involvement of other hormones, such as androgens, E_2, or 17β-diol, may explain the discrepancy, because as detailed below, many of these steroids can also produce altered affect.

The relative insensitivity to steroid potentiation of GABA-gated current described above may not occur under all instances of sustained increases in circulating levels of progesterone. For example, the mood-stabilizing effects of progesterone during pregnancy do not exhibit tolerance, possibly as a result of the presence of these other psychotropic hormones. Although this topic deserves further study, use of a pseudopregnancy paradigm, which enables the ovary to release high levels of progesterone and thus 3α,5α-THP into the circulation, in the absence of other hormones such as E_2, has in fact been shown to result in tolerance to the anxiolytic effects of 3α,5α-THP (D. B. Bitran, personal communication).

Other neuroactive/psychoactive steroids

DHEA and DHEAS

In the adult, DHEA is formed primarily by the adrenal from 17α-hydroxypregnenolone, where it can function as a steroid hormone precursor as well as a neuroactive steroid [31, 79]. In normal women, circulating levels of this steroid and its sulfated form, DHEAS, are in the micromolar range [80]. Conflicting reports exist in terms of a role for this steroid in PMS or PMDD [5, 80]. DHEAS has been shown to antagonize GABA receptor- mediated current [81], assessed both in recombinant receptor systems [3] and in studies measuring inhibitory synaptic activity [82–84]. In other studies, this steroid was shown to augment excitatory NMDA responses in the CA3 region of rat dorsal hippocampus via a selective action on the sigma-1 receptor [34]. No effects on acetylcholine or quisqualate responses were observed.

There are conflicting reports of behavioural DHEAS effects. It can be anxiogenic [85]. In one study [86], a case of adrenal hyperplasia resulted in an anxiety syndrome, which was best attenuated by reduction in DHEAS formation. Other studies, however, have described DHEAS as having anxiolytic, antidepressant, and antiaggressive properties [87, 88]. The reason for this discrepancy may be dose-related: i.e., optimal levels of the steroid are anxiolytic, but above a threshold they become anxiogenic. Alternatively, there may be differences in the relative conversion of DHEAS to other positive modulators of the $GABA_A$ receptor or to other psychoactive androgens, such as testosterone. Under some conditions, DHEAS may be more slowly metabolized and thus act directly as a negative GABA modulator when it would be

anxiogenic. Ambient conditions which favor conversion of this steroid to metabolites which are positive modulators of the $GABA_A$ receptor would then be expected to yield anxiolytic properties.

More globally, DHEAS increases neuronal excitability, enhances neuronal plasticity, and has neuroprotective properties [79]. In addition, this compound has memory-enhancing and antiamnestic effects [89].

Pregnenolone sulfate

Pregnenolone sulfate is a steroid hormone precursor in both the periphery and CNS, where it also functions as a potent modulatory steroid. It was the first neurosteroid to be described by Jung-Testas et al. [90], and it fluctuates in many cases in parallel with progesterone, increasing to maximal levels during the luteal phase of the menstrual cycle. In the CNS, pregnenolone sulfate is a positive modulator of NMDA actions [91], with inhibitory actions on the GABAR complex [84, 92] primarily mediated through a decrease in Cl^- channel open frequency. Behaviorally, this steroid enhances learning and memory [93]. Effects on mood have not yet been established. Although not usually evaluated in PMS studies, one recent report [94] has described elevations in this steroid across both phases of the menstrual cycle in women suffering from mixed anxiety-depressive disorder.

Pre-menstrual syndrome

Comparison with the progesterone exposure and withdrawal rat model of PMS

The clinical literature is filled with sometimes contradictory reports of PMS etiology. However, a recent conference [4] devoted to discussion of this disorder has concluded that it is a distinct clinical entity to be differentiated from classic affective disturbances. There are probably at least two predominant points in the menstrual cycle when adverse symptomatology can occur: (i) during the hormonal decline in the late luteal phase (PMS) and (ii) as an abnormal response to hormone exposure shortly after the mid-cycle peak in ovarian hormones (PMDD).

With regard to PMS occurring in the late luteal phase, there is a body of evidence that hormone 'withdrawal' can be correlated with dysphoria. Throughout the past several decades, progesterone has been suggested as a mediating factor, although this has been under dispute more recently. A more likely candidate is the GABA-modulatory $3\alpha,5\alpha$-THP, because this compound is anxiolytic and can display withdrawal properties to produce PMS-like anxiogenic states. A number of studies support the hypothesis that declining levels of steroid increase anxiety. Several studies have suggested an inverse correlation between circulating levels of $3\alpha,5\alpha$-THP and adverse symptomatology either in the same individual or across groups [95, 96]. However, others have failed to find such a relationship, but have noted that adverse symptomatology can be associated with declining levels of the hormone [6, 97]. One recent study [98] has reported that in women with a history of post-partum depression, hormone 'withdrawal' precipitates adverse symptomatology suggesting that other underlying factors govern the response to hormonal fluctuations. This possibility is also suggested by an earlier study correlating an increased risk of PMS symptomatology in women with pre-existing affective disorder [99]. Replacement therapy with either $3\alpha,5\alpha$-THP or progesterone can improve symptomatology [100], but often not significantly better than placebo, thus making it difficult to reach a definite conclusion. One study noted that treatment with micronized progesterone did not attenuate the craving for chocolate and sweets sometimes associated with PMS [101]. Recent studies report that $3\alpha,5\alpha$-THP treatment does not produce any change in the hypothalamo-pituitary-gonadal axis, including E_2, luteinizing hormone, follicle-stimulating hormone, and prolactin levels, nor are the pharmacokinetics of this hormone altered in women with PMS [102], suggesting that any beneficial effects of the compound are more likely to be related to its known GABA-modulatory effect.

For PMDD, adverse symptomatology commences shortly after the mid-cycle peak in ovarian hormones and persists throughout the luteal phase, thus precluding hormone 'withdrawal' in the etiology. Some recent innovative studies have explored the hypothesis that absolute levels of the steroid do not produce adverse symptoms but, rather, PMDD may simply be triggered by hormone exposure. In the study conducted by Schmidt and colleagues [103], PMDD symptomatology was effectively prevented by terminating cyclicity with the LHRH agonist leuprolide. Under these conditions, only PMDD-prone women, but not control subjects, responded to short-term hormone treatment with dysphoria, suggesting that PMDD is the result of an abnormal response to steroids in susceptible women. Similarly, several studies [105] have reported higher levels of circulating E_2 [104, 105] and progesterone in PMDD sufferers. The hypothesis that PMDD-prone women have a different response to steroids has been further investigated by Backstrom and Sundstrom. Their studies have revealed that women with PMDD exhibit slower eye saccade velocity [106], a measure of GABAergic tone, during the luteal phase, but are markedly less responsive to the modulatory effect of benzodiazepines [69] and neurosteroids [107] on this end-point. Their work suggests that PMDD-susceptible women have increased GABAergic tone during the luteal phase, a finding difficult to explain, but consistent with the idea that there may be increased GABA modulation by endogenous factors in relevant CNS circuits, including neuroactive steroids [107].

The relative benzodiazepine insensitivity observed is consistent with an increase in the α4 GABAR subunit, and suggests that similar changes in GABAR subunit composition may occur across the menstrual cycle in PMDD-susceptible women as observed in our rodent model. The second effect, i.e., insensitivity to 3α,5α-THP, is also one of the pharmacological changes observed in our PMS model [64], which may be due to changes in other GABAR subunits. Necessarily, however, this model fails to predict the prolonged period of dysphoria in PMDD patients, which may be a function of other receptor systems or hormones, as discussed below.

Other PMS hormone or drug correlations

Estradiol

In addition to progesterone and its metabolites, E_2 levels have been correlated with PMS symptomatology [105]. In one recent study [104], increased levels of both E_2 and the pituitary hormone luteinizing during the luteal phase were well correlated with the severity of negative premenstrual symptoms. In this study, PMS symptomatology developed shortly after ovulation, reaching maximal levels during the late luteal phase, during the hormone 'withdrawal' period. Typically, symptoms abated several days after the onset of menses. E_2 has been associated primarily with excitatory effects on the CNS [36] and on affect [13], which may be experienced as anxiety and irritability.

In contrast, in other groups of women, estrogen 'withdrawal' may also be a factor, as suggested by several studies demonstrating that administration of estrogen can have anti-depressant effects [108–110]. The anti-depressant effect of estrogen was described in early clinical studies, and more recently as a treatment strategy for depressive symptoms associated with PMDD [111], the post-partum period [108] as well as peri-menopausally [109]. These paradoxical effects of E_2 may be related to the predisposition of the individual involved, or interactions with other neuroactive hormones.

Androgens

Testosterone may be produced by the adrenal or ovary in women where it functions as the immediate precursor for E_2, following conversion catalyzed by the aromatase enzyme, which can occur in the periphery or within the CNS. Circulating levels of testosterone fluctuate across the menstrual cycle, reaching maximal levels at mid-cycle. Exogenous treatment with testosterone may produce improvements in mood. In one study [112], a group of oophrectomized women receiving transdermal testosterone reported improved well-being and decreased depressive symptomatology compared to their pre-treatment values. Although reports are conflicting [80], a recent study has reported lower total and free testosterone plasma levels in women with PMDD in the absence of changes in adrenal steroids [113]. Thus, the level of this androgen may be one factor triggering the subtype of premenstrual dysphoria which has an onset in the early luteal phase.

Melatonin

A recent study has described defective melatonin cycles during the luteal phase in women with PMS [114], which include a delay in melatonin onset time, compressed duration, with decreases in integrated area, amplitude, and mean levels. Exposure to bright light ameliorated symptomatology, and was associated with significant changes in melatonin rhythm. Because melatonin has been associated with anti-depressant actions, altered circadian rhythm of this hormone may be related to depressive symptomatology in PMS-susceptible women.

Selective serotonin reuptake inhibitors

The selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac), sertraline, and citalopram are effective in 60% of patients treated for PMDD [4]. Several studies [115–119] have demonstrated that intermittent treatment of PMDD-susceptible women with SSRIs alleviates adverse symptomatology more consistently than other treatments. Interestingly, this effect is noted after a faster onset and with lower doses of these drugs than required for their classic anti-depressant effects, suggesting a mechanism distinct from the serotonin receptor system which requires prolonged exposure to SSRIs. In addition, classic tricyclic anti-depressant drugs do not effectively ameliorate PMDD dysphoria [117]. Although serotonergic effects on mood have been well-documented, the recently reported effect of this compound in stimulating synthesis of neuroactive steroids in the CNS may be the mechanism underlying its ameliorative effect on PMDD symptomatology [120]. In vitro studies have reported that SSRIs directly increase synthesis of 3α,5α-THP from progesterone by decreasing the K_m of 3α-HSD activity by 10- to 30-fold below control values [121]. A recent clinical study has also demonstrated that fluoxetine administration to depressive patients significantly increases the cerebrospinal fluid (CSF) content of 3α,5α-THP in close association with improvement in depression self-rating, as assessed using the Hamilton Rating Scale [122]. In contrast, CSF levels of pregnenolone and progesterone levels were not altered by fluoxetine treatment, while levels of the GABA-enhancing adrenal steroid 3α,5α-tetrahydrodeoxycorticosterone were decreased by fluoxetine treament [123], suggesting a role for 3α,5α-THP in mediating the mood-altering effect of this psychotropic

drug. Although partial tolerance to the GABA-modulatory effect of 3α,5α-THP is observed in the rodent model of PMDD, with elevated levels of this steroid produced by the SSRI, enhancement of GABA-mediated inhibition in limbic circuits could have a significant behavioral effect. In fact, a clinical study [118] has demonstrated that citalopram administration increases pregnanolone sensitivity in women with PMS, assessed using eye saccade velocity as a measure of its GABA-potentiating effect.

Conclusions

It is clear from a review of the literature that complex hormonal changes occur across the menstrual cycle. In addition to their clearly established effects on the hypothalamo-pituitary-gonadal axis, many of these steroids or their metabolites exert potent effects on classic neurotransmitter systems. The most well-known of these are the GABA-modulatory steroids, of which 3α,5α-THP is the most potent. The homeostatic response of the GABAergic system to prolonged exposure and 'withdrawal' from this steroid may be similar to its response to conventional GABA-modulatory drugs such as the benzodiazepines and alcohol. That is, upon chronic exposure and/or withdrawal from this steroid, GABAR subunit composition is altered, primarily through an increase in the α4 subunit, which then decreases GABA-generated current, thereby decreasing inhibition within CNS circuits of the limbic system. These changes could then precipitate anxiety in susceptible individuals, or even exacerbate ongoing seizure activity in epileptic individuals ('catamenial epilepsy'). However, hormone 'withdrawal' may only explain one subtype of PMS characterized by dysphoria during the late luteal decline in progestins. A second subtype of PMS, PMDD, which is triggered by hormone exposure may also involve 3α,5α-THP, but must also be mediated by an abnormal response to other hormones such as E_2 or to lowered levels of hormones such as testosterone. Necessarily, however, the picture is also complicated by individual differences in GABAR sensitivity, effects of other steroids and inherent differences in susceptibility to affective changes. A continuing investigation of steroid effects on CNS circuits is crucial for our understanding of the effect of endogenous hormonal fluctuations on human behavior.

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