Cellular and molecular effects of steroid hormones on CNS excitability

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ABSTRACT

The steroid hormones 17β-estradiol (estradiol) and progesterone not only regulate the reproductive system but have other central nervous system effects that can directly affect a variety of behaviors. Generally, estradiol has been shown to have activating effects, including the ability to increase seizure activity, while progesterone has been shown to have depressant effects, including anticonvulsant properties. Because levels of these hormones fluctuate across the menstrual cycle, it is important to understand how changes in these hormone levels may influence levels of excitability in the brain, especially in women who have seizure patterns that are related to their menstrual cycle, a phenomenon known as catamenial epilepsy. This paper reviews the effects of estradiol and progesterone on excitatory and inhibitory neurotransmitters, respectively, and the possible cellular and molecular mechanisms underlying the changes in brain excitability mediated by these hormones.

In addition to their well-known effects on reproductive actions mediated through classic nuclear receptors, the ovarian hormones 17β-estradiol (estradiol) and progesterone can also exert nonclassic effects on the central nervous system (CNS) that alter a variety of behaviors. Estradiol has been shown to have activating effects on mood (euphoria, anxiety, or antidepressant effects), cognition, sensory response, motor behavior, and seizure activity.[1,2] Progesterone produces effects that are generally opposite to those produced by estradiol.[1] Increases in circulating levels of progesterone have been correlated with depressant effects, including anxiolytic[3] and anticonvulsant[4,5] effects. At higher doses, this hormone is sedative and can act as a general anesthetic,[6] an effect first demonstrated by Hans Selye in the 1940s.[7]

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Hormones and epilepsy across the menstrual cycle

Despite the fact that these hormones have very well-characterized effects on nuclear receptors, many of these nontraditional effects in the brain may be due to nonclassic actions of the hormones on conventional neurotransmitters. Estradiol and progesterone, or their metabolites, acutely potentiate responses to excitatory (estradiol) or inhibitory (progesterone metabolites) neurotransmitters in a rapid fashion (seconds to minutes) and, after chronic exposure or withdrawal (days to weeks), produce structural, synaptic, or molecular effects by which both hormone systems increase CNS excitability.

Across the menstrual cycle, estradiol is elevated in the second half of the follicular phase and increases to a peak at midcycle, while progesterone is primarily elevated during the luteal phase and declines before menstruation begins. The contrasting effects of these hormones in activating or depressing CNS function, respectively, may have implications for behavior or perhaps even epilepsy across the cycle.

Catamenial epilepsy is a change in seizure frequency or severity across the menstrual cycle;[8] increases in seizure severity have been reported at the midcycle peak in ovarian hormones and also during the late luteal phase, during the decline in ovarian hor-

mones—a period that may be a time of hormone withdrawal.

THE BASIS OF ESTRADIOL'S EXCITATORY EFFECTS

One cellular mechanism contributing to the excitatory effects of estradiol is its ability to rapidly increase responses of neurons to the excitatory neurotransmitter glutamate **(Figure 1A)**.[9–11] Glutamate, in turn, can activate a number of receptor subtypes, including those selective for AMPA and kainate, the typical glutamate receptors responsible for fast synaptic transmission at excitatory synapses in the brain. These receptors are composed of four subunits, and once bound, the transmitter gates open a channel that allows Na^+ in to depolarize the neuron, thereby increasing its activity. The NMDA-selective subtype of glutamate receptor, however, requires extensive depolarization before channel gating occurs, owing to a Mg^{2+} block that is unblocked by depolarization. The NMDA receptor is permeable to both Na^+ and Ca^{2+}. The Ca^{2+} influx that accompanies NMDA channel activation may contribute to neuronal plasticity, as well as neural degeneration under excessive activation, as is sometimes seen during seizure states.

This potentiating effect of estradiol on excitatory synaptic transmission influences both the non-NMDA[12–15] and the NMDA[16] types of glutamate receptors, the former due to a G-protein–dependent mechanism involving protein kinase A activation.[13] This rapid effect of estradiol may underlie the observation that direct application of estradiol to the cortex of an animal can produce de novo ictal discharges.[17]

In contrast to estradiol's rapid effect, more prolonged exposure to estradiol results in structural and functional changes at excitatory synapses that selectively enhance neurons' sensitivity to NMDA receptor–mediated synaptic input. These effects have been mostly studied in the hippocampus, which is often a site for the initiation and propagation of limbic seizure activity. Excitatory synapses on neurons in the hippocampus are formed by presynaptic axonal varicosities, from which neurotransmitter vesicles are released, and postsynaptic dendritic spines, which are small thornlike protrusions that densely cover the dendrites of neurons.

Anatomic studies in animals such as rats have shown that 3 days' exposure to elevated estradiol levels increases the number and density of dendritic

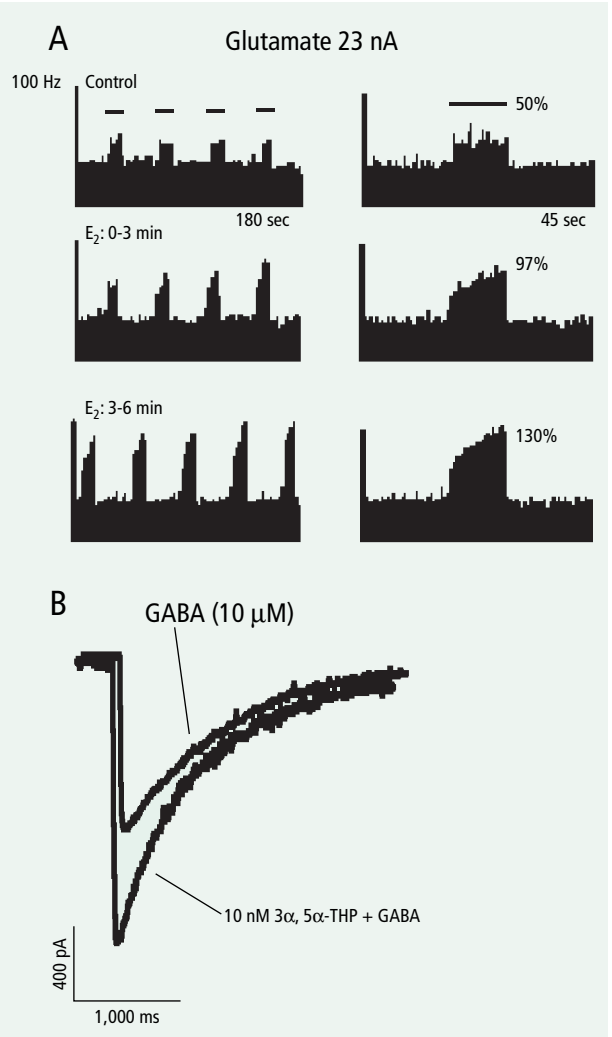

FIGURE 1. Acute application of **(A)** 17β-estradiol or **(B)** a progesterone metabolite exerts opposite effects on neuronal responses to neurotransmitters. **(A)** Local acute application of 17β-estradiol (E_2) increases cerebellar Purkinje cell responses to iontophoretically applied glutamate, an excitatory transmitter (bars above histogram). Both individual responses (left) and averaged response (right) of extracellular discharge from a representative neuron are presented. **(B)** In contrast, physiologic concentrations of the progesterone metabolite 3α-OH-5α-pregnan-one (THP) increase GABA-gated current recorded from acutely isolated CA1 hippocampal pyramidal neurons using whole cell patch-clamp recording techniques.

spines **(Figure 2)** and excitatory synapses on hippocampal neurons.[18] Notably, increases in the number or density of spines and synapses occur not only with estradiol treatment but also as hormone levels fluctuate naturally across the reproductive cycle.[18] Further anatomic analysis of axonal varicosities has shown that estradiol increases the number of vari-

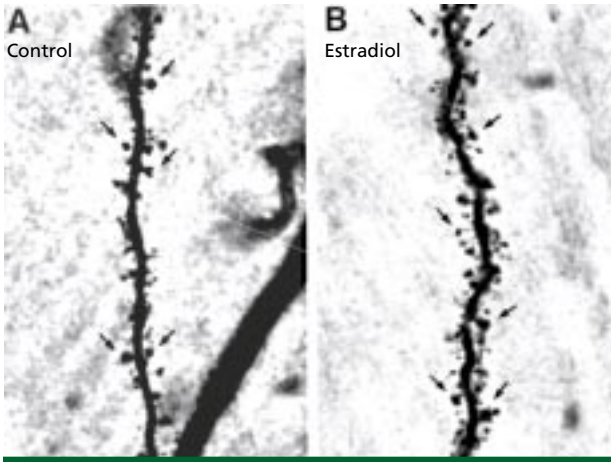

FIGURE 2. Estradiol increases dendritic spine density, as shown in these photomicrographs of representative dendrites on hippocampal neurons from **(A)** a control female rat that was ovariectomized to remove endogenous ovarian hormones and **(B)** an ovariectomized female rat treated for 3 days with estradiol. Note that the density of dendritic spines, small thornlike protrusions that are sites of excitatory synaptic contact, is greater in the estradiol-treated rat. Some dendritic spines are indicated by arrows. Reprinted, with permission, from reference 21. Copyright 1997 by the Society for Neuroscience.

cosities that make synaptic connections with multiple dendritic spines, and that these multiple spines arise from different postsynaptic neurons.[19]

Thus, anatomic studies show that estradiol not only increases the density of excitatory inputs to individual neurons in the hippocampus but also promotes divergence of pre- to postsynaptic input. This change could increase the synchronization of synaptically driven neuronal firing in the hippocampus, and therefore may be important in estradiol's proconvulsant effects on limbic seizure activity.

Because the synapses formed on dendritic spines are glutamatergic, the anatomic data described above predict that estradiol would increase neuronal sensitivity to glutamatergic synaptic input. Indeed, electrophysiologic studies show this to be the case. Interestingly, estradiol selectively increases neuronal sensitivity to synaptic input mediated by the NMDA type of glutamate receptor, while responses mediated by the AMPA receptor are not affected **(Figure 3)**.[20] This electrophysiologic result is corroborated by receptor-binding autoradiography studies showing that estradiol increases glutamate binding to NMDA, but not AMPA, receptors[21] and histologic studies indicating that estradiol increases expression of the NMDA receptor subunit that is common to all forms of the NMDA receptor.[22]

Because NMDA receptors have been shown to be important in experimental models of epilepsy, this functional effect of estradiol also could contribute to its proconvulsant effects. Consistent with this prediction, estradiol has been shown to increase hippocampal seizure susceptibility in several animal models: direct measurement of electrographic seizure threshold in the hippocampus,[23] hippocampal kindling,[24] and chemically induced seizures that depend upon the hippocampus, such as kainate-induced behavioral seizures.[2]

THE BASIS OF PROGESTERONE'S DEPRESSANT EFFECTS

Another ovarian hormone, progesterone, can exert numerous effects via a classic nuclear receptor, but can also exert effects via nonnuclear receptors after it is readily metabolized via two enzymatic conversions in the brain to a neuroactive steroid, 3α-OH-5α-pregnan-one (3α,5α-THP, or allopregnanolone). The primary effect of 3α,5α-THP and its isomer, 3α,5β-THP, is to modulate the $GABA_A$ receptor,[25] which mediates most fast inhibition in the brain **(Figure 1B)**. Levels of this metabolite in the circulation parallel those of progesterone; therefore, it is increased during the luteal phase and during pregnancy.

The $GABA_A$ receptor is a pentameric structure composed of varying combinations of 5 subunits from a pool of 17 genetically distinct subunit subtypes: 6 α, 3 β, 3 γ, and 1 each of δ, ε, π, θ, and ρ.[26] Each subunit, in turn, is composed of 4 membrane-spanning α-helices, with the second transmembrane segment surrounding a central chloride channel. Generally, this receptor contains 2 α, 2 β, and 1 γ subunits, but other combinations exist. Different subunit isoforms can produce receptors with varying biophysical and pharmacologic properties.

When two molecules of GABA bind to the $GABA_A$ receptor, the central chloride channel is gated open, allowing Cl^- influx into the neuron, which hyperpolarizes most neurons of the adult CNS and results in inhibition of neuronal activity. The $GABA_A$ receptor is also the target of most known depressant sedative drugs, such as benzodiazepines, barbiturates, and anesthetics, which all bind to unique sites on the receptor, as does the steroid 3α,5α-THP. At physiologic concentrations, this steroid rapidly enhances the ability of GABA to allow Cl^- into the cell[25] by increasing the open time of the channel;[27] as a result, this steroid is more

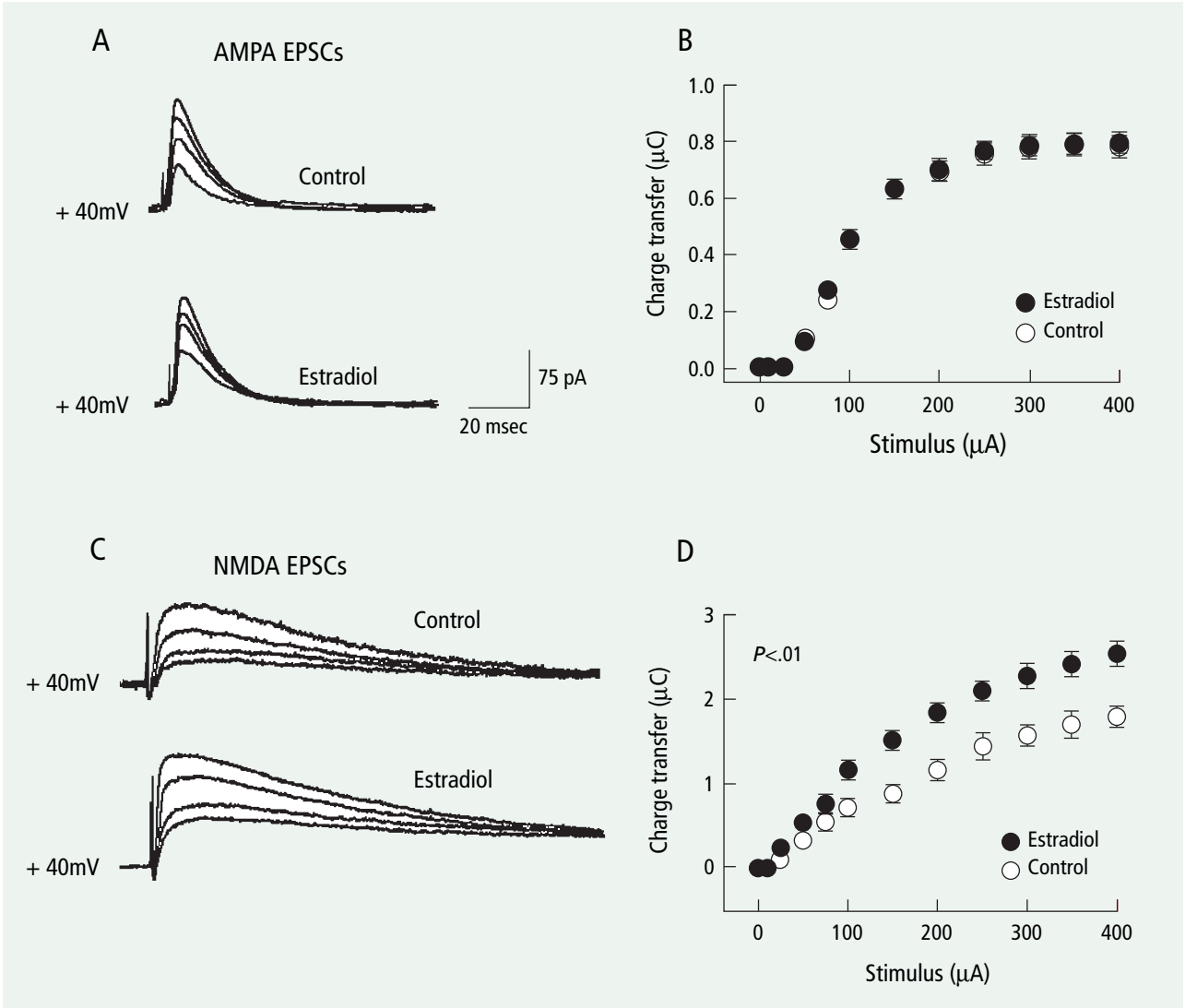

FIGURE 3. Estradiol increases neuronal sensitivity to glutamatergic synaptic input mediated by the NMDA type of glutamate receptor, with no effect on the AMPA type. Shown are electrophysiologic recordings of AMPA **(A)** and NMDA **(C)** receptor–mediated excitatory postsynaptic currents (EPSCs) in hippocampal neurons from control and estradiol-treated animals, and stimulus-response curves for AMPA **(B)** and NMDA **(D)** receptor–mediated neuronal responses. Note that estradiol treatment increases neuronal sensitivity to NMDA receptor–mediated input, with no effect on AMPA receptor–mediated responses. Hormone treatment was identical to that of Figure 1, and AMPA and NMDA responses were recorded from the same cells. Adapted, with permission, from reference 20. Copyright 2001 by the Society for Neuroscience.

potent as an anxiolytic than the benzodiazepine class of tranquilizers, and in fact can act effectively as an anxiolytic, anticonvulsant, and even anesthetic drug.

Progesterone withdrawal and CNS excitability

Across the menstrual cycle, circulating levels of this steroid are increased for 10 to 12 days before declining to low levels. It is therefore important to characterize not only acute effects but also chronic and potential withdrawal effects of the steroid on $GABA_A$ receptor function. Such withdrawal effects are seen with other sedative drugs, such as alcohol. Using a 21-day-administration paradigm in rats, withdrawal from 3α,5α-THP resulted in a behavioral excitability state characterized by increased anxiety and seizure activity triggered by $GABA_A$ channel blockers.[28–30] Across the entire time course of progesterone exposure, a more complex pattern of anxiety behavior has emerged,[31] with anxiety levels increasing after 48 to 72 hours of exposure, then

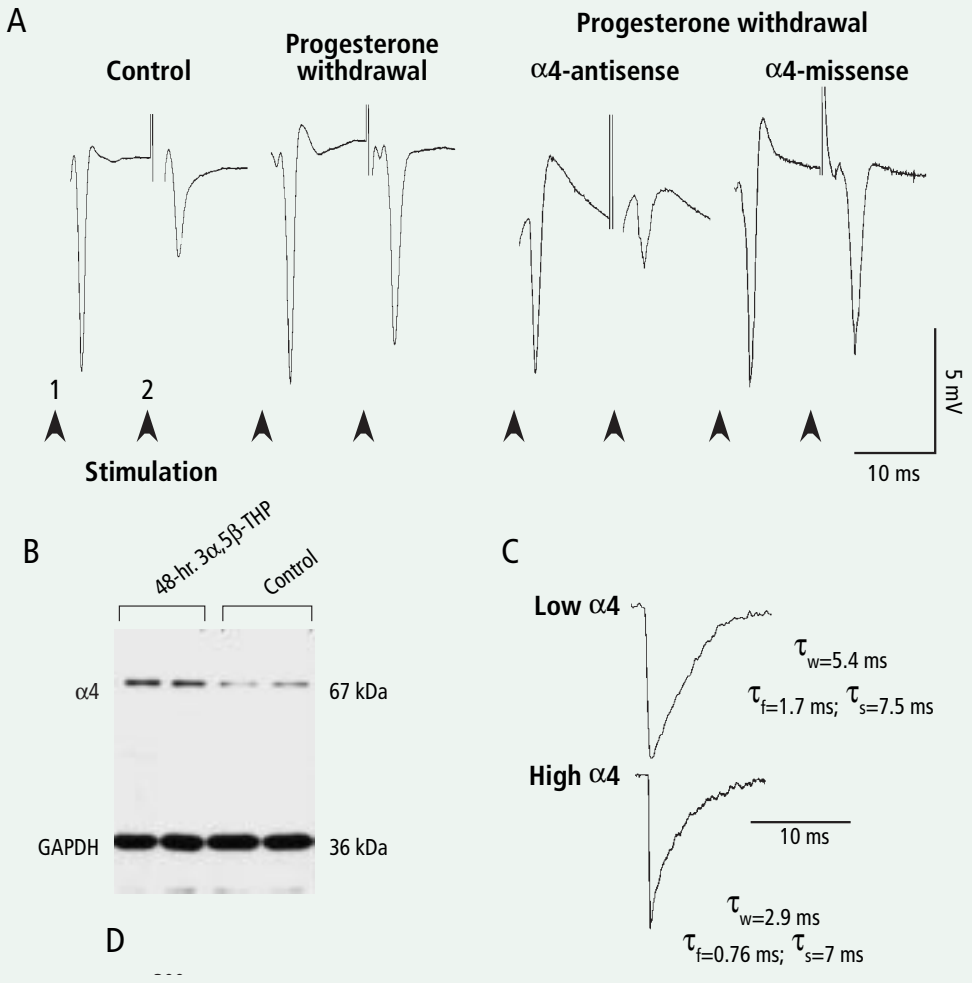

FIGURE 4. Progesterone withdrawal increases CNS excitability, an effect dependent upon $GABA_A$ receptor α4 subunit upregulation. **(A)** Paired-pulse inhibition is a model of hippocampal circuit excitability, such that response to the second of two paired stimuli ("2") is smaller than response to the first ("1"). This inhibition is reduced following progesterone withdrawal (ie, response "2" is larger). This effect was prevented when increases in α4 subunit expression were suppressed by intraventricular administration of α4 antisense oligonucleotide during withdrawal, but not altered in the missense control. Reprinted, with permission, from reference 34. **(B)** Chronic exposure to the GABA-modulatory progesterone metabolite 3α,5β-THP for 48 hours increases expression of the $GABA_A$ receptor α4 subunit similar to progesterone withdrawal (67-kDa band on the representative Western blot). **(C)** Suppression of α4 expression during 48-hour steroid treatment ("Low α4") produced inhibitory synaptic current with a slower decay than normally observed after steroid treatment ("High α4"), suggesting that increased α4 expression produces reduced inhibition. Reprinted, with permission, from reference 33. **(D)** Seizure activity produced by the Cl^- channel blocker picrotoxin is increased after progesterone withdrawal, an effect prevented when α4 expression is suppressed by antisense treatment during the withdrawal period. These results suggest that chronic treatment and withdrawal from progesterone and its 3α,5β-THP metabolite result in increased excitability, both in vitro and in vivo, because of increased expression of $GABA_A$ receptors containing the α4 subunit. Adapted, with permission, from reference 30. Copyright 1998 Nature Publishing Group.

decreasing by 5 to 7 days after exposure until withdrawal, when anxiety again increases. This bimodal pattern of anxiety response is, in fact, similar to the pattern reported in catamenial epilepsy,[8] with exacerbation of seizures reported at midcycle and again during the late luteal-phase decline in circulating levels of progestins.

A similar pattern of change is observed when the cellular characteristics of hippocampal neurons are analyzed. Both 2-day progesterone exposure and progesterone withdrawal result in GABA-gated current nearly insensitive to modulation by benzodiazepines, owing to an increase in expression of novel subtypes of $GABA_A$ receptors containing the α4 subunit,[30,31] which are uniquely insensitive to modulation by the benzodiazepine class of GABA modulators.[32]

The increase in α4 expression also leads to decreases in inhibition gated by the $GABA_A$ receptor, as suggested by several findings **(Figure 4)**. First, suppression of α4 expression using antisense technology prevents the increase in seizure susceptibility observed following progesterone withdrawal.[30] Under conditions of suppressed α4 expression, measures of reduced inhibition seen at the circuit and synaptic level following progesterone withdrawal are also prevented.[33,34] At the circuit level, inhibitory feedback triggered by paired stimuli (paired-pulse inhibition) is significantly attenuated following progesterone withdrawal.[34] At the synaptic level, unitary current recorded from the CA1 region of the hippocampus after 48-hour 3α,5α/β-THP exposure exhibits a faster decay than control. If the total integrated current is evaluated after hormone exposure, there is a reduction in the total amount of Cl^- transferred, leading to a reduction in inhibitory tone.[33] Because suppression of α4 expression prevents these effects, these findings suggest that substitution of novel α4-containing $GABA_A$ receptors for the ambient receptor population after progesterone exposure/withdrawal leads to reduced inhibition in the brain, thus permitting increased CNS excitability.

SUMMARY AND CONCLUSIONS

Across the menstrual cycle, it appears that both the acute and chronic effects of estradiol enhance excitatory input around the time of the midcycle peak. In contrast, the acute effects of the progesterone metabolite appear to enhance inhibitory responses of limbic neurons during the luteal phase, until the time of hormone decline, when altered $GABA_A$ receptors' subunit composition would reduce inhibition, leading again to increased excitability **(Figure 5)**. Thus, these diverse effects of ovarian hormones tend to exacerbate seizure activity at midcycle and during the late luteal phase, a pattern common to catamenial epilepsy.

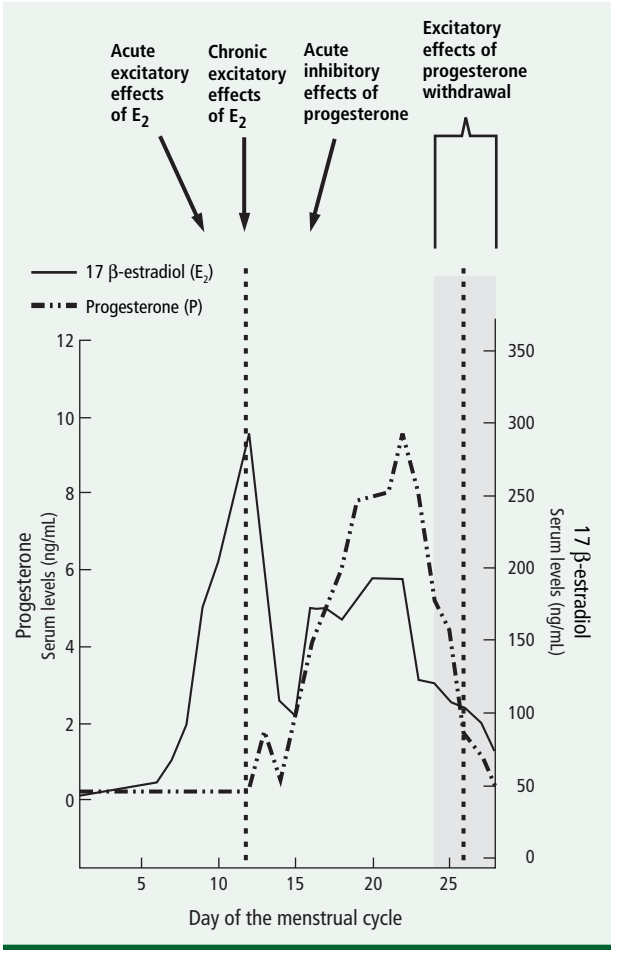

FIGURE 5. Potential time course of altered excitability by ovarian steroids across the menstrual cycle. Time course for fluctuations in circulating levels of progesterone (left axis) and 17β-estradiol (E_2, right axis) during a typical 28-day menstrual cycle. The first two arrows indicate theoretical time points when acute and chronic actions of E_2 might exert excitatory effects on the CNS via glutamate receptors and increases in excitatory synapse formation, respectively, around the midluteal peak in levels of this hormone. In contrast, potentiation of GABA-mediated inhibition by 3α,5α-THP during the progesterone-dominant luteal phase (third arrow) would produce a potentially anticonvulsant effect until the decline in steroid levels ("withdrawal," shaded area), when decreased inhibition may result as a function of lower 3α,5α-THP levels and the formation of quickly decaying α4-containing $GABA_A$ receptors. Dotted lines indicate time points for exacerbation of seizure activity associated with catamenial epilepsy.

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