

DANCING THE DELTA SHUFFLE: NEUROSTEROIDS REGULATE GABA_A RECEPTOR EXPRESSION

Ovarian Cycle-linked Changes in GABA(A) Receptors Mediating Tonic Inhibition Alter Seizure Susceptibility and Anxiety

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Disturbances of neuronal excitability changes during the ovarian cycle may elevate seizure frequency in women with catamenial epilepsy and enhance anxiety in premenstrual dysphoric disorder (PMDD). The mechanisms underlying these changes are unknown, but they could result from the effects of fluctuations in progesterone-derived neurosteroids on the brain. Neurosteroids and some anxiolytics share an important site of action: tonic inhibition mediated by δ subunit-containing GABA_A receptors (δ GABA_ARs). Here we demonstrate periodic alterations in specific GABA_AR subunits during the estrous cycle in mice,

causing cyclic changes of tonic inhibition in hippocampal neurons. In late diestrus (high-progesterone phase), enhanced expression of δ GABA_ARs increases tonic inhibition, and a reduced neuronal excitability is reflected by diminished seizure susceptibility and anxiety. Eliminating cycling of δ GABA_ARs by antisense RNA treatment or gene knockout prevents the lowering of excitability during diestrus. Our findings are consistent with possible deficiencies in regulatory mechanisms controlling normal cycling of δ GABA_ARs in individuals with catamenial epilepsy or PMDD.

Short-term Steroid Treatment Increases δ GABA_A Receptor Subunit Expression in Rat CA1 Hippocampus: Pharmacological and Behavioral Effects

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In this study, 48-h administration of 3α -OH- 5β -pregnan-20-one ($3\alpha,5\beta$ -THP) or 17β -estradiol (E_2)+progesterone (P) to female rats increased expression of the δ subunit of the GABA_A receptor in CA1 hippocampus. Coexpression of $\alpha 4$ and δ subunits was suggested by an increased response of isolated pyramidal cells to the GABA agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP), following 48-h steroid treatment, and nearly complete blockade by 300 μ M lanthanum (La^{3+}). Because $\alpha 4\beta\delta$ GABA_A receptors are extrasynaptic, we also recorded pharmacologically isolated GABAergic holding current from CA1 hippocampal pyramidal cells in the slice. The La^{3+} -sensitive THIP current, representative of current gated by $\alpha 4\beta\delta$ GABA_A receptor, was measurable only following 48-h

steroid treatment. In contrast, the bicuculline-sensitive current was not altered by steroid treatment, assessed with or without 200 nM gabazine to block synaptic current. However, 48-h steroid treatment resulted in a tonic current insensitive to the benzodiazepine agonists lorazepam (10 μ M) and zolpidem (100 nM). These results suggest that 48-h steroid treatment increases expression of $\alpha 4\beta\delta$ GABA_A receptors, which replace the ambient receptor population. Increased anxiolytic effects of THIP were also observed following 48-h steroid treatment. The findings from the present study may be relevant for alterations in mood and benzodiazepine sensitivity reported across the menstrual cycle.

COMMENTARY

GABA_A receptors are the primary mediators of inhibitory signaling in the brain, making them important targets for therapeutic drugs. One such class of GABA_A receptor

modulators, the neurosteroids, can be either excitatory or inhibitory, depending on the particular steroid and GABA_A receptor isoform (1,2). Classically, steroid hormones are thought to regulate gene transcription by interacting with nuclear hormone receptors. In contrast, neurosteroids interact directly with GABA_A receptors independently of the nuclear hormone receptors (3), and, therefore, research has focused on the acute effects of neurosteroids on brain physiology. Chronic treatment with other GABA_A receptor modulators, such as benzodiazepines or alcohol, causes a state of behavioral tolerance, in part as a result of altered GABA_A receptor expression (4,5). Although tolerance is less likely to occur with neurosteroids, prolonged neurosteroid administration does alter the function and expression of GABA_A receptors (6). Endogenous neurosteroid synthesis occurs in the periphery as well as within the brain. Shifts in neurosteroid production are evident over the course of the ovulatory cycle. The studies presented here explore the exciting possibility that, in addition to their well-known and immediate modulation of GABA_A receptors, neurosteroids may have long-term effects that regulate inhibitory neurotransmission.

Women of reproductive age exhibit dramatic changes in ovarian hormones during the course of the menstrual cycle. Following the midcycle hormone surge and subsequent ovulation, there is a period of 1 to 2 weeks in which the corpus luteum produces large amounts of progesterone (luteal phase). As the corpus luteum begins to fail, progesterone levels fall, thereby signaling the onset of menses and the end of the reproductive cycle. Progesterone has acute sedating and antiepileptic properties, largely as a result of its conversion to neurosteroids via the actions of 3 α -hydroxysteroid dehydrogenase and 5 α -reductase in various tissues. The abrupt decline in progesterone/neurosteroids is thought to be one of the reasons that many women with epilepsy have increased seizure frequency immediately prior to menses.

In an effort to clarify the relationship between menstrual changes and seizure frequency, hormonal manipulations to rodent models of epilepsy are often used. However, there are important differences between the ovulatory cycles of rodents and primates. In rats, a hormone surge occurs on the day of proestrus, followed by ovulation and a period of sexual receptivity (estrus) that is accompanied by low estrogen and progesterone levels. Then, similar to the primate luteal phase, there is a brief period of rising progesterone (diestrus). However, unlike the human luteal phase, diestrus lasts for only 2 to 3 days; thus, the cycling rodent does not have the prolonged period of progesterone exposure, which is a key feature of the primate menstrual cycle.

Some rodent models of chronic progesterone have included "pseudopregnancy" or administration of exogenous progesterone to more closely model premenstrual changes in women

(2). Although acutely administered progesterone and neurosteroids are sedating, prolonged progesterone exposure in some models results in a brief period of increased anxiety behavior and benzodiazepine resistance. After a few days, these changes resolve, but subsequent progesterone withdrawal causes a rebound hyperexcitable state, with increased anxiety and propensity toward seizures. These changes are not due to progesterone itself, but rather to its conversion to neurosteroids. Chronic progesterone treatment results in elevated levels of 3 α -hydroxy-5 α -pregnan-20-one (3 α , 5 α -THP), or allopregnanolone, in the blood and CNS (7). Furthermore, prolonged application of either allopregnanolone or 3 α , 5 β -THP (pregnanolone) mimics the effects of chronic progesterone. Finally, inhibition of 3 α -hydroxysteroid dehydrogenase or 5 α -reductase, which prevents the conversion of progesterone to allopregnanolone, abolishes the effects of chronic progesterone exposure.

The cellular basis for this neurosteroid-induced hyperexcitable state remains unknown. However, subacute (2 to 4 days) neurosteroid exposure in rats is associated with increased hippocampal $\alpha 4$ GABA_A receptor expression, which returns to baseline levels with prolonged neurosteroid treatment. On neurosteroid withdrawal, there is a powerful rebound increase in $\alpha 4$ GABA_A receptor subunit expression. The hyperexcitability induced by both subacute neurosteroids and neurosteroid withdrawal can be blocked with intracerebroventricular injection of antisense $\alpha 4$ mRNA. During these hyperexcitable states, inhibitory postsynaptic currents (IPSCs) in CA1 neurons are shortened in duration and are benzodiazepine insensitive yet can be enhanced by the $\alpha 4/6\beta\gamma$ -selective imidazobenzodiazepine compound Ro 15-4513. Since recombinant $\alpha 4\beta 2\gamma 2$ GABA_A receptors deactivate more quickly than those containing $\alpha 1\beta 2\gamma 2$, it has been suggested that synaptic $\alpha 1\beta\gamma$ GABA_A receptors may be replaced by $\alpha 4\beta\gamma$ GABA_A receptors during hyperexcitable states (8).

In addition, $\alpha 4$ proteins can combine with other GABA_A receptor subunits, such as the δ subunit. Neurosteroids (including pregnanolone and allopregnanolone) are especially powerful modulators of $\alpha\beta\delta$ -containing GABA_A receptors. These neurosteroids increase the maximal GABA-evoked current several fold and convert $\alpha\beta\delta$ GABA_A receptor responses to highly desensitizing, slowly deactivating currents (9). Furthermore, in some brain regions, the $\alpha\beta\delta$ -containing GABA_A receptors are thought to reside outside the synapse where they are nearly continuously exposed to low levels of GABA. Although, the tonic currents conveyed by this population of GABA_A receptors are small, their long duration allows them to convey a very large overall charge transfer. The studies presented here test the hypothesis that progesterone/neurosteroid exposure alters inhibitory signaling in the CNS by regulating the expression of $\alpha\beta\delta$ -containing GABA_A receptors in specific brain regions.

The paper by Shen et al. explores the effects of subacute (2 days) progesterone or neurosteroid treatment on GABA_A receptor expression in CA1. These studies found increased levels of $\alpha 4$ and δ proteins, with more modest reductions in the expression of $\alpha 1$ and $\gamma 2$ proteins. Since $\alpha\beta\delta$ GABA_A receptors convey the tonic current in some brain areas, the authors tested the hypothesis that steroid treatment would alter the tonic current in CA1 hippocampal neurons. Although neurosteroid treatment did not alter amplitude of the tonic current, there did appear to be a change in the relative contribution of different GABA_A receptor isoforms. The CA1 tonic current in control rat brain slices is largely conveyed by $\alpha 5\beta\gamma$ GABA_A receptors (10). So application of benzodiazepine-like compounds, including lorazepam (enhances $\alpha 1,2,3,5\beta\gamma$), zolpidem (enhances $\alpha 1\beta\gamma$ and to a lesser extent $\alpha 2,3\beta\gamma$), and Ro 15-4513 (enhances $\alpha 4,6\beta\gamma$), were used to test for the relative contribution of various $\alpha\beta\gamma$ GABA_A receptors. Unlike control slices, the tonic currents from neurosteroid-treated rats were insensitive to all three agents, suggesting that the contribution of all γ -containing GABA_A receptors to the tonic current was reduced. 4,5,6,7-Tetrahydroisoxazolo-[5,4-c]-pyridine-3-ol (THIP), also known as gaboxadol, is a partial agonist at most GABA_A receptors. However, this compound is a superagonist at $\alpha 4\beta\delta$ GABA_A receptors, with a maximal response that is greater than the response to GABA. Following neurosteroid treatment, the whole cell currents from steroid-treated cells were more sensitive to THIP than GABA. Furthermore, although the trivalent heavy metal lanthanum (La³⁺) interacts with several different receptors and ion channels, its effects on GABA_A receptors are relatively selective. While La³⁺ often enhances GABA_A receptor currents, low concentrations of La³⁺ act as a selective inhibitor of $\alpha 4\beta\delta$ GABA_A receptors. As might be expected with increased $\alpha 4\beta\delta$ GABA_A receptor expression, the large THIP-induced current was almost completely blocked by La³⁺, while cells from control slices were relatively insensitive. Taken together, the authors make a compelling argument that subacute neurosteroid treatment alters the composition of GABA_A receptor subtypes that convey the tonic current in hippocampal neurons.

The paper by Maguire et al. takes the findings of Shen and colleagues a step further and explores the regulation of GABA_A receptor expression over the ovulatory cycle. This study found that hippocampal δ GABA_A receptor subunit expression in mice is increased and $\gamma 2L$ GABA_A receptor expression is reduced during a period of elevated progesterone (late diestrus), compared with hippocampal GABA_A receptor subunit expression during a low-progesterone period (estrus). Unlike exogenous neurosteroid treatment, $\alpha 4$ GABA_A receptor expression was not altered. To explore the physiologic significance of these changes, electrophysiological recordings were made in hippocampal brain slices taken from mice during estrus and

compared with those from mice in late diestrus. There was no difference in the amplitude, frequency, or decay time of IPSCs in either CA1 or dentate granule neurons. Moreover, similar to the finding by Shen et al., there was no change in tonic current amplitude in CA1 neurons. However, in dentate granule cells, in which the tonic current is largely conveyed by $\alpha\beta\delta$ GABA_A receptors (11), the amplitude of the tonic current was enhanced during diestrus. These cellular changes were associated with behavioral evidence of reduced anxiety and a lower propensity for seizures. Altered δ expression appeared to play a causative role in these changes, as there was increased sensitivity to the antiepileptic effects of THIP. Furthermore, experiments with intracerebroventricular injection of antisense δ mRNA or using δ knockout mice confirmed that the tonic current in dentate gyrus granule cells is carried largely by $\alpha\beta\delta$ -containing GABA_A receptors, and that enhanced expression of the δ subunit is required for the seizure resistance seen during diestrus.

A strength of the paper by Maguire and colleagues is that it addresses GABA_A receptor function as it relates to the reproductive cycle. However, control of the ovulatory cycle involves complex interactions among hormones and CNS function. It should be noted that other than measuring blood progesterone levels, the Maguire et al. study did not specifically test whether the observed changes were actually caused by progesterone or neurosteroids. Since both the Maguire and Shen et al. studies used reproductively intact animals, it is entirely possible that the altered GABA_A receptor expression in cycling animals is independent of progesterone/neurosteroids and involves some other component of the reproductive axis. The involvement of another regulatory system may explain some of the discrepancies in the findings between the two papers. In contrast to the Shen et al. study, the cycling mice utilized in the Maguire et al. study did not have the elevated $\alpha 4$ GABA_A receptor expression, shortened IPSCs, or behavioral hyperexcitability seen during subacute neurosteroid treatment in rats.

An alternate interpretation of these discrepant findings is that the concentration and duration of progesterone/neurosteroid exposure may be crucial in regulating GABA_A receptor expression. It is unclear how long the progesterone levels were elevated in the mice described in the Maguire paper, but previous hormone profile studies would suggest that the exposure probably only lasted a few hours to a day. During prolonged neurosteroid exposure and withdrawal, there is also increased expression of $\alpha 4$ containing GABA_A receptors. Elevated expression of both $\alpha 4$ and δ GABA_A receptor subunits could certainly alter the relative proportion of $\alpha 1\beta\delta$ -, $\alpha 4\beta\delta$ -, $\alpha 1\beta\gamma$ -, and $\alpha 1\beta\gamma$ -containing GABA_A receptors in the hippocampus. GABA_A receptors containing $\alpha 4\beta\delta$ subunits are sensitive to low levels of GABA, and $\alpha 4\beta\gamma$ GABA_A receptors deactivate quickly after brief GABA applications. However, the

response of these receptors to prolonged or repetitive GABA exposure remains unknown, and so it is unclear how the transient hyperexcitable state caused by subacute neurosteroid treatment and withdrawal is actually related to altered GABA_A receptor expression.

Maguire et al. associate the increased δ subunit expression in cycling mice with enhanced tonic current amplitude and seizure resistance. However, it should be kept in mind that δ -containing GABA_A receptors are particularly sensitive to modulation by neurosteroids. It is unlikely that residual gonadal steroids are present in the brain slices used for electrophysiological recordings. In contrast, it is possible that the local production of neurosteroids continues in hippocampal slices and that the capacity to synthesize neurosteroids varies over the estrous cycle, thereby contributing to the altered tonic current measured by Maguire et al. Future studies will need to determine whether modulation of neurosteroid synthetic enzymes allow a level of control over CNS excitability throughout the ovarian cycle.

The studies presented here show that in addition to acute allosteric effects, neurosteroids may regulate CNS excitability by altering the expression of their target GABA_A receptors. It will be important to further define the time course and regional specificity of neurosteroid regulation of GABA_A receptor expression. Hormone levels change quickly over the reproductive cycle, and the response to those hormones may be equally fluid. Just as important, many of the changes in GABA_A receptor expression described here are similar to those seen in some models of epilepsy. An active area of research involves exploring the possibility that similar regulation of certain GABA_A receptor isoforms is involved in epileptogenesis. The studies reviewed here help highlight the fine temporal and spatial specificity of CNS excitability and provide insights into the exquisite choreography controlling the ballet of brain function in health and disease.

by Andre Lagrange, MD, PhD

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