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The Perimenstrual Delta Force: A Trojan Horse for Neurosteroid Effects

Perimenstrual-Like Hormonal Regulation of Extrasynaptic δ-Containing GABAA Receptors Mediating Tonic Inhibition and Neurosteroid Sensitivity.

Carver CM, Wu C, Gangisetty O, Reddy DS. *J Neurosci* 2014;34(43):14181–14197

Neurosteroids are endogenous regulators of neuronal excitability and seizure susceptibility. Neurosteroids, such as allopregnanolone (AP; 3α-hydroxy-5α-pregnan-20-one), exhibit enhanced anticonvulsant activity in perimenstrual catamenial epilepsy, a neuroendocrine condition in which seizures are clustered around the menstrual period associated with neurosteroid withdrawal (NSW). However, the molecular mechanisms underlying such enhanced neurosteroid sensitivity remain unclear. Neurosteroids are allosteric modulators of both synaptic (αβγ2-containing) and extrasynaptic (αβδ-containing) $GABA_A$ receptors, but they display greater sensitivity toward δ-subunit receptors in dentate gyrus granule cells (DGGCs). Here we report a novel plasticity of extrasynaptic δ-containing $GABA_A$ receptors in the dentate gyrus in a mouse perimenstrual-like model of NSW. In molecular and immunofluorescence studies, a significant increase occurred in δ subunits, but not α1, α2, β2, and γ2 subunits, in the dentate gyrus of NSW mice. Electrophysiological studies confirmed enhanced sensitivity to AP potentiation of GABA-gated currents in DGGCs, but not in CA1 pyramidal cells, in NSW animals. AP produced a greater potentiation of tonic currents in DGGCs of NSW animals, and such enhanced AP sensitivity was not evident in δ-subunit knock-out mice subjected to a similar withdrawal paradigm. In behavioral studies, mice undergoing NSW exhibited enhanced seizure susceptibility to hippocampus kindling. AP has enhanced anticonvulsant effects in fully kindled wild-type mice, but not δ-subunit knock-out mice, undergoing NSW-induced seizures, confirming δ-linked neurosteroid sensitivity. These results indicate that perimenstrual NSW is associated with striking upregulation of extrasynaptic, δ-containing $GABA_A$ receptors that mediate tonic inhibition and neurosteroid sensitivity in the dentate gyrus. These findings may represent a molecular rationale for neurosteroid therapy of catamenial epilepsy.

Commentary

Hormones influence neuronal excitability and seizure susceptibility in both adulthood and during development (1–8). A paradigm illustrating these interactions is catamenial epilepsy, where seizures may cluster around specific periods of the menstrual cycle (3). The perimenstrual (C1) type of catamenial epilepsy is a type of epilepsy in which seizures cluster around the menstrual phase when estradiol, progesterone, as well as the neurosteroid allopregnanolone decline. Allopregnanolone is a progesterone metabolite known to act on $GABA_A$ receptors. Progesterone is converted to 5a-dihydroprogesterone (5α-DHP) by 5α-reductase, and then 5α-DHP is converted to allopregnanolone by 3β-hydroxysteroid dehydrogenase (3β-HSD).

Reddy's team has been a pioneer in studies aiming to shed light in the molecular and pathophysiological mechanisms of increased seizure susceptibility in perimenstrual epilepsy. To recreate the hormonal and neurosteroid changes occurring during this perimenstrual period, they introduced a model of neurosteroid withdrawal (NSW) in adult female mice by administering high dose progesterone twice daily for 7 days followed by finasteride, a 5α-reductase inhibitor that blocks neurosteroid synthesis (8). This NSW model recreates high levels of allopregnanolone during the progesterone administration phase, which rapidly decline to normal levels during NSW. Interestingly, NSW mice demonstrate increased susceptibility to kindling associated with increased expression of the α4 $GABA_A$ receptor subunit in the dentate gyrus granule cells (8). The Carver et al. study completes these findings by showing a parallel increased expression of the δ $GABA_A$ receptor subunit, a common partner of α4 in extrasynaptic $GABA_A$ receptors, as well as enhanced allopregnanolone potentiation of tonic but also phasic $GABA_A$ receptor currents in NSW mice. Such findings would be normally considered as capable of evoking antiseizure effects by increasing tonic inhibition. However, the authors found smaller tonic GABA current shifts in NSW granule cells compared to controls in the absence of added GABA, possibly suggesting the existence of silent $GABA_A$ receptors or low extracellular GABA in NSW hippocampus. In δ knock-out

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mice (δKO), however, NSW did not affect seizures, and there was no allopregnanolone sensitivity suggesting that these effects are $GABA_A$ receptor dependent. Cyclic or hormonal state dependent changes in δ or α4 subunits have previously been demonstrated in normal cycling mice (9) or in other models of progesterone withdrawal (10), although the effects on seizure susceptibility may vary. Such differences may be due either to model or method specific effects or to the study-related differences in hormonal and neurosteroid environment. The existing studies have been done, thus far, in mice that do not have spontaneous seizures, and it would certainly be important to extend this NSW model in a rodent model of epilepsy.

The NSW experimental model is a classical illustration that a rapid shift in homeostatic balance, rather than a specific range of allopregnanolone levels, can be pathogenic. Progesterone treatment increases allopregnanolone without altering $GABA_A$ receptor mRNA expression (8). Conversely, NSW mice demonstrate allopregnanolone levels similar to vehicle but with significantly increased extrasynaptic $GABA_A$ receptor subunits α4 and δ (8, 11) and seizure susceptibility. How does the brain sense this homeostatic change and adopt patterns of signaling that are defined by recent homeostatic changes? The authors previously provided evidence that NSW induces early growth response factor 3 (Egr3), leading to upregulation of the α4 subunit and increasing seizure susceptibility (8). The exact signaling pathway that leads to upregulation of δ subunits is less clear. It would be interesting to explore how this NSW-mediated regulation of Egr3, α4, and δ subunits is controlled by the rapidity or magnitude of allopregnanolone decline and what is the upstream regulatory trigger for this effect.

The authors are commended for their careful evaluation of the signaling pathways that are necessary for the NSW effects, via use of specific knock-out mice and antisense approaches. Using progesterone receptor knock-out mice (PRKO), they exclude the possibility that intracellular progesterone receptors A and B are involved, and shift their focus on $GABA_A$ receptors, a well known target of allopregnanolone. Yet, progesterone and allopregnanolone signaling can be complex. Both progesterone and allopregnanolone can activate membrane progesterone receptors (mPR), G-protein coupled membrane receptors that may inhibit adenylyl cyclase activity having anti-apoptotic effects and also regulatory effects on pulsatile gonadotropin releasing hormone (GnRH) secretion (12). Among other signaling effects, the inhibition of adenylyl cyclase could in turn further inhibit the transduceosome, a mitochondrial protein complex that includes translocase (TSPO; former peripheral benzodiazepine receptor). TSPO is the rate-limiting step in the mitochondrial conversion of cholesterol to pregnenolone, a precursor of progesterone. Could mPR-mediated inhibition of TSPO and mitochondrial progesterone and neurosteroid synthesis render neurons more dependent upon exogenous neurosteroids during NSW? It would be interesting to explore this idea in future studies.

The focus on allopregnanolone effects on $GABA_A$ receptors is, however, justified here by the demonstration that δKO mice or antisense δ knockdown in the hippocampus blunt the electrophysiological and seizure susceptibility effects of NSW and alllopregnanolone. However, allopregnanolone has additional effects that could affect hippocampal pathology and function. For example, pregnane X receptors (PXR; intracellular transcriptional factors) and the mitochondrial permeability transition pore (MPTP) are well-known mediators of anti-apoptotic and anti-inflammatory effects of allopregnanolone. Activation of PXR by allopregnanolone exerts neuroprotective effects in a Niemann–Pick disease model (13), although this effect is $GABA_A$ receptor independent. Egr3, an immediate early response gene shown to be increased in NSW mice, has been implicated in adaptation to stress and novelty as well as long-term depression (14). Could allopregnanolone also affect the anxiety or depression associated with perimenstrual symptoms or perimenstrual epilepsy? A randomized double-blind, placebo-controlled clinical study of pregnenolone in individuals with bipolar disorder indeed shows antidepressive effects that correlate negatively with the effects on plasma allopregnanolone (15). If this extends to the perimenstrual dysphoric symptoms, it would be an additional benefit of such treatment.

Another interesting finding is the regional selectivity of NSW and allopregnanolone effects for dentate granule cells versus CA1 pyramidal neurons. Dentate granule cells are well known as gatekeepers of seizure propagation to the hippocampus, which explains the observed effects of NSW and allopregnanolone on kindling-induced seizures. Despite the systemic changes in plasma allopregnanolone levels during NSW and allopregnanolone treatment, neurosteroid synthesis is largely dependent upon local synthesis within neurons that may create a different neurosteroid concentration in various brain regions. Indeed, this in situ de novo synthesis of hormones or neurosteroids has been proposed to explain the sexual dimorphism of selected brain regions. Such findings caution against generalizing these mechanisms to all brain regions or over-relying on plasma levels when local hormone or neurosteroid concentrations could be more accurate.

In essence, the proposed mechanism poses that the perimenstrual decline in neurosteroids triggers the selective overexpression of α4δ subunits in the dentate gyrus granule cells, but this is not sufficient to suppress the increased seizure susceptibility of NSW mice in the absence of allopregnanolone. Yet, this pathologic increase in α4δ subunits may act as a vehicle-catalyst ("Trojan horse") for the exogenous allopregnanolone to inhibit seizures. It is, therefore, a beautiful example of utilizing a pathogenic disease feature to deliver an effective therapy. Indeed, this special sensitivity (dependence) to allopregnanolone that women with perimenstrual epilepsy exhibit is in agreement with the findings from the NIH Progesterone Trial, in which the responder group was women with the C1 type of catamenial epilepsy (16). Interestingly, these women responders demonstrated significant posttreatment increase in allopregnanolone levels (17). This scenario would render perimenstrual epilepsy an ideal candidate to test the therapeutic effects of allopregnanolone as a brief pulse treatment protocol during this perimenstrual period. However, the optimization of the clinical treatment protocol would still need to be carefully thought out to avoid unwanted effects on the hypothalamus–pituitary axis, such as inhibition of gonadotropins and amenorrhea (18) or side effects after treatment interruption, such as resurgence of depression, anxiety or breakthrough seizures. Yet, studies such as the one by Carver et al. help unravel the target mechanisms that could be relevant

to those promoting perimenstrual seizures and facilitate the implementation of target-directed treatment protocols that could be safer and more effective.

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Erratum

In the article "The Perimenstrual Delta Force: A Trojan Horse for Neurosteroid Effects" [Epilepsy Currents, Vo.l 15, No. 2 (March/April) 2015 pp.80-82, the first sentence of the last paragraph, should read: "In essence, the proposed mechanism poses that the perimenstrual decline in neurosteroids triggers the selective overexpression of $\alpha 4\delta$ subunits in the dentate gyrus granule cells, but this is not sufficient to suppress the increased seizure susceptibility of NSW mice in the absence of allopregnanolone."

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