Potentiating the Strength of Extrasynaptic Currents by Neurosteroid Hormones

Neurosteroid Structure-Activity Relationships for Functional Activation of Extrasynaptic δGABA$_A$ Receptors.

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Synaptic GABA$_A$ receptors are primary mediators of rapid inhibition in the brain and play a key role in the pathophysiology of epilepsy and other neurologic disorders. The δ-subunit GABA$_A$ receptors are expressed extrasynaptically in the dentate gyrus and contribute to tonic inhibition, promoting network shunting as well as reducing seizure susceptibility. However, the neurosteroid structure-function relationship at δGABA$_A$ receptors within the native hippocampus neurons remains unclear. Here we report a structure-activity relationship for neurosteroid modulation of extrasynaptic GABA$_A$ receptor–mediated tonic inhibition in the murine dentate gyrus granule cells. We recorded neurosteroid allosteric potentiation of GABA as well as direct activation of tonic currents using a wide array of natural and synthetic neurosteroids. Our results shows that, for all neurosteroids, the C3α-OH group remains obligatory for extrasynaptic receptor functional activity, as C3β-OH epimers were inactive in activating tonic currents. Allopregnanolone and related pregnane analogs exhibited the highest potency and maximal efficacy in promoting tonic currents. Alterations at the C17 or C20 region of the neurosteroid molecule drastically altered the transduction kinetics of tonic current activation. The androstane analogs had the weakest modulatory response among the analogs tested. Neurosteroid potentiation of tonic currents was completely (approximately 95%) diminished in granule cells from δ-knockout mice, suggesting that δ-subunit receptors are essential for neurosteroid activity. The neurosteroid sensitivity of δGABA$_A$ receptors was confirmed at the systems level using a 6-Hz seizure test. A consensus neurosteroid pharmacophore model at extrasynaptic δGABA$_A$ receptors is proposed based on a structure-activity relationship for activation of tonic current and seizure protection.

Commentary

In adult mammalian neurons, ionotropic GABA$_A$ receptors function as inhibitory channels, facilitating the entry of negatively charged chloride ions into the cell. This inhibitory effect occurs in the presence of GABA—the endogenous ligand for GABA$_A$ receptors, and also other molecules, such as neurosteroids, which act as positive allosteric modulators. GABA$_A$ receptors are pentameric structures comprising a mixture of subunit proteins (to date including α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3 proteins) that form a central ion channel. This impressive diversity results in a vast array of potential combinations of receptor species, each with different functional properties, cell-surface distributions, and regional expression patterns within the brain. GABA$_A$ receptors containing a δ subunit are primarily located extrasynaptically, where they mediate a tonic form of inhibition, which is temporally unrelated to phasic synaptic events (1). GABA-mediated tonic conductance (I_{tonic}) has been identified in several brain regions, notably the dentate gyrus (2), where it modulates network excitability in the hippocampus. Given the function and the regional location of these receptors, it is not surprising that they have been closely linked with epilepsy: antagonists of GABA$_A$ receptors, such as bicuculline, promote seizures, whereas activators of these receptors, such as diazepam and stiripentol (3), are used clinically as antiseizure agents. In addition, mutations in *Gabrd*, the gene coding for the δ subunit of the GABA$_A$ receptor, appear to contribute to epilepsy susceptibility (4).

Neuroactive steroids, or neurosteroids, act as positive allosteric modulators of GABA$_A$ receptors, potently enhancing the inhibitory effects of GABA (5). While a detailed understanding of how these molecules interact with GABA$_A$ receptors to modulate channel function has been generated for synaptic γ-containing receptors (6), structure-activity relationships of neurosteroids acting at extrasynaptic receptors has not been established. This represents critical missing information, since δ-subunit–containing GABA$_A$ receptors appear preferentially sensitive to physiological GABA concentrations (7), and neurosteroids exhibit reduced tonic current and decreased sensitivity in δ-subunit knock-out (δKO) mice (8). The authors therefore set out to confirm the influence of the δ-subunit of GABA$_A$ receptors for GABA-mediated tonic current, and to characterize the structure-activity relationship and functional consequences of neurosteroids acting at δ-containing GABA$_A$ receptors on I_{tonic}.

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First, using dissociated dentate gyrus granule cell neurons from wild-type (WT) and δ-subunit KO mice, they show that the potentiating effects of allopregnanolone (AP), a well-studied neurosteroid, on GABA-mediated inhibition is dramatically reduced in the absence of δ-subunits, reaffirming previous reports of the preferential sensitivity for neurosteroids at δ-containing $GABA_A$ receptors. They then moved to hippocampal slice preparations obtained from female WT and δKO mice in diestrus I stage. This consistency is important since variation in endogenous levels of steroid hormones, and consequently neurosteroids, and of δ-containing $GABA_A$ receptors, occurs throughout the ovarian cycle (9). Measurement of tonic current was achieved using the patch-clamp technique, removing synaptic currents by treatment with the sodium channel inhibitor TTX. GABA (0.3–10 μm) dose-dependently increased I_{tonic} in WT slices, but only the highest concentration (10 μm) increased tonic current in slices from δKO mice, which is beyond physiological GABA concentrations. Then, in the presence of 1 μm GABA, AP dose-dependently enhanced I_{tonic}, and this was far greater in WT slices than in δKO slices, supporting the results generated from the dissociated neuron prep.

They next assessed a library of endogenous and synthetic neurosteroid compounds for their ability to potentiate I_{tonic} using WT slices. This allowed the rank order of potency of this collection of steroids to be established, and advances on most other research which typically investigates pregnane-derived neurosteroids, such as AP or Tetrahydrodeoxycorticosterone (THDOC), in isolation. Furthermore, varying specific components of the base neurosteroid structure facilitated structure-activity relationships to be generated for the neurosteroid scaffold. They found that, in general, the pregnane neurosteroids were more potent than the androstane-derived compounds, but that all (with one exception) neurosteroids consistently potentiated the effects of GABA on I_{tonic}. In the next series of studies, the authors set out to establish whether the previously reported seizure-suppressing effects of neurosteroids (10) were related to the potentiation of extrasynaptic $GABA_A$ receptor-mediated tonic current. To test this, they employed the 6-Hz model of electrically evoked seizures and studied a range of doses to establish the ED_{50} for each of the neurosteroids from in the cellular assays. Most of the neurosteroids tested suppressed seizures in the model dose-dependently, achieving 100% protection. Notably, the only compound that failed to achieve complete seizure suppression was 3β5α-AP, and this was also the only compound that elicited no potentiation of $GABA_A$-receptor–mediated tonic current, even at high concentrations. In addition, the rank order of potency of the compounds was very similar between seizure suppression and potentiation of tonic current, strongly supporting that this in vivo measure is directly related to the electrophysiological action of neurosteroids.

Because the research covered a range of related naturally occurring and synthetic compounds, the authors summarized the results of their studies by developing a pharmacophore map describing the critical components that dictate the structure-functional activity relationship of neurosteroids at extrasynaptic $GABA_A$ receptors. Five components of the structure were identified that dictate the efficacy and potency of neurosteroids. Of note, the C3α-OH group of the neurosteroid remained essential for functional activity at extrasynaptic receptors, whereas alterations of the C17 or C20 regions influenced the kinetics of tonic current activation. In the future, these components can be selectively manipulated to develop new tools to study allosteric modulation of this receptor, and to identify novel therapeutic agents targeting epilepsy. Indeed, one of the synthetic neurosteroids investigated in the study is Ganaxolone, the most potent compound in the 6-Hz anticonvulsant assay, and a drug which is currently in the end-stages of clinical trials for epilepsy.

by Nigel C. Jones, PhD

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