Sometimes Steroids Get a Bad Rap

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Seizure Progression Is Slowed by Enhancing Neurosteroid Availability in the Brain of Epileptic Rats

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Trilostane is a 3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase inhibitor able to produce a manyfold increase in brain levels of various neurosteroids, including allopregnanolone. We previously found that treatment with trilostane can slow down epileptogenesis in the kainic acid (KA) model of temporal lobe epilepsy. It is unknown whether trilostane may have a similar effect on the progression of epilepsy severity, as observed in KA-treated rats. Consequently, we investigated the effects of trilostane (50 mg/kg/day, 1 week) in epileptic rats, given 64 days after KA administration. Seizures were monitored by video-electrocorticographic recordings before and during the treatment with trilostane or vehicle (sesame oil), and neurosteroid levels were measured in serum and cerebral tissue using liquid chromatography-electrospray tandem mass spectrometry after treatment. Pregnenolone sulfate, pregnenolone, progesterone, 5α-dihydroprogesterone, and allopregnanolone peripheral levels were massively increased by trilostane. With the only exception of hippocampal pregnenolone sulfate, the other neurosteroids augmented in both the neocortex and hippocampus. Only pregnanolone levels were not upregulated by trilostane. As expected, a significant increase in the seizure occurrence was observed in rats receiving the vehicle, but not in the trilostane group. This suggests that the increased availability of neurosteroids produced a disease-modifying effect in the brain of epileptic rats.

Commentary

A significant focus of epilepsy research is the development of new therapies that target GABA receptor function.[1] Of particular interest is the role of neurosteroids. Neurosteroids are endogenous compounds manufactured in the brain, specifically by principal, excitatory neurons by converting cholesterol through a series of reactions to generate allopregnanolone (AP) and tetrahydrodeoxycorticosterone (THDC).[2] These compounds have been shown to act allosterically to modulate neuronal excitability through interactions with the $GABA_A$ receptor.[3] Low concentrations of neurosteroids have been shown to enhance $GABA_A$ receptor function while higher concentrations of these compounds can directly open $GABA_A$ receptor channels to decrease neuronal excitability.[3] Neurosteroid regulation of $GABA_A$ receptor function appears to be mediated through an autocrine action with the neurosteroid diffusing within the lipid of the cell membrane to interact with both synaptic and extrasynaptic $GABA_A$ receptors to affect both phasic and tonic inhibition.[3,4]

While this suggests that increasing the levels of neurosteroids in the brain could be an effective antiepileptogenic or disease-modifying therapy, it also suggests that a decrease in neurosteroid levels in the brain could be proepileptic. Consistent with this observation, finasteride, a drug that inhibits the production of AP, has been shown to enhance seizure susceptibility in an animal model of premenstrual catamenial epilepsy.[5] A clinical evaluation of neurosteroid levels in the CSF of patients during status epilepticus (SE) revealed a significant decrease in AP which suggests that a reduction in AP could contribute to the seizure activity observed after SE.[6] A reduction in the rat hippocampal concentration of AP was found to correlate with seizure frequency after kainic acid (KA)-induced SE.[7] These findings support the development of neurosteroids or drugs that enhance their production as a potential therapies for pharmacoresistant epilepsy.

The highlighted report[8] is a culmination of a series of studies by Biagini and colleagues designed to test the effect of the compound trilostane on different metrics of epileptogenesis and spontaneous recurrent seizure (SRS) frequency in the KA rat model of SE.[9,10] Trilostane is a reversible inhibitor of 3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase that increases the levels of neurosteroids in the brain. Trilostane was found to increase levels of pregnenolone, progesterone, 5α-dihydroprogesterone, and AP in the hippocampus and neocortex when administered to naïve rats.[9] Pretreatment with trilostane before inducing SE with KA had no effect on the latency to the onset

of SE or its duration but resulted in a faster onset and offset of stage 4/5 behavioral seizures, with individual seizure duration shorter than in vehicle controls.[9] A subsequent study examined the effect of repeated administration of trilostane on epileptogenesis after SE.[10] Trilostane administration initiated 10 minutes after KA and continued for 6 days during the latent period had no effect on the severity of SE and was not neuroprotective. However, this treatment with trilostane significantly delayed the onset of spontaneous recurrent electrographic and behavioral seizures suggesting that increasing the levels of neurosteroids during the latent period after SE had an antiepileptogenic effect.[10]

The current study was designed to determine the effect of trilostane on SRS progression after KA-induced SE.[8] In the KA model of SE, after the onset of SRS there is a progressive increase in seizure frequency over time eventually reaching a plateau.[11] Trilostane (50 mg/kg) dissolved in sesame oil was injected subcutaneously once/day for 1 week starting 64 days after KA-induced SE in male Sprague Dawley rats. Controls animals were injected with vehicle. The timing of the trilostane injections was selected because it corresponded with the rising phase of SRS frequency.[11] SRS were detected by video/EEG monitoring initiated before the trilostane injections. The animals were euthanized 6 hours after the last trilostane injection and neurosteroid levels were measured in the sera, hippocampi, and neocortices by liquid chromatography tandem mass spectrometry. While treatment with trilostane had no effect on the severity or duration of SRS it did prevent the progressive increase in seizure frequency observed in vehicle controls. This decrease in SRS progression was accompanied by a significant elevation in several steroids in the sera, hippocampus, and neocortex. However, pregnanolone was not elevated in the hippocampus or neocortex and the hippocampus differed from the sera and neocortex in that trilostane did not induce an increase in hippocampal pregnenolone sulfate. The trilostane-induced elevation of neurosteroid precursors in the blood is relevant since they could potentially contribute to an increase in neurosteroid production in brain tissue. The observed elevation of AP in both the hippocampus and neocortex after treatment with trilostane is significant given AP's reported action of enhancing $GABA_A$ receptor function and could be mediating the observed decrease in seizure progression.

The authors interpreted the trilostane-induced slowing of the increase in SRS frequency after SE as an indication that the trilostane-induced increase in the levels of neurosteroids in the brain had a disease-modifying action. However, additional experiments are needed to determine if the post SE increase in SRS frequency returns if treatment with trilostane is discontinued and to determine the effect of trilostane on SRS frequency once the plateau phase is reached. This is important because the observed decrease in seizure frequency progression after treatment with trilostane could be an acute, anti-seizure effect. It would also be interesting to determine the effect of trilostane on the elevation of SRS frequency if it were administered before or immediately after the appearance of the first spontaneous seizure. Finally, it is important to test the effect of trilostane in other models of epilepsy. Allopregnanolone and THDC have been tested in several experimental models with evidence that these agents can interfere with epileptogenesis, interictal spiking, and high-frequency oscillations.[12] However, these results tend to be model and timing dependent. This may be due to the observation that in some models, $GABA_A$ receptor expression is altered, especially in models of SE where there can be modification of receptor subunit composition and a loss of receptors with δ subunits which mediate tonic inhibition and are one of the targets of neurosteroids.[12]

In conclusion, the results from this study provide additional evidence that treatments that increase the production of cortical and hippocampal neurosteroids have the potential to be both antiepileptogenic and disease modifying and warrant further development as a therapy for pharmacoresistant seizures. Currently ganaxolone, an analogue of AP, is the only neurosteroid clinically approved for the treatment of epilepsy; specifically for seizures associated with CDKL5 deficiency disorder.[1]

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Declaration of Conflicting Interests

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References

1. Perucca P, Bialer M, White HS. New GABA-targeting therapies for the treatment of seizures and epilepsy: I. Role of GABA as a modulator of seizure activity and recently approved medications acting on the GABA system. *CNS Drugs*. 2023;37(9):755-779. doi:10.1007/s40263-023-01027-2
2. Agis-Balboa RC, Pinna G, Zhubi A, et al. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *PNAS*. 2006;103(39):14602-14607. doi:10.1073/pnas.0606544103
3. Reddy DS, Rogawski MA. Neurosteroids-regulators of seizure susceptibility and role in the treatment of epilepsy. In: Noebles JL, Avoli M, Rogawski AM, Olsen RW, Delgado-EscuetaAV, eds. *Jasper's Basic Mechanisms of the Epilepsies*. 4th ed. National Center for Biotechnology Information (US) 2012: 1135-1156.
4. Chisari M, Eiseman LN, Covey DF, Mennerick S, Zorumski CF. The sticky issue of neurosteroids and $GABA_A$ receptors. *Trends Neurosci*. 2010;33(7):299-306. doi:10.1016/j.tins.2010.03.005
5. Reddy DS, Kim HY, Rogawski MA. Neurosteroid withdrawal model of perimenstrual catamenial epilepsy. *Epilepsia*. 2001; 42(3):328-336. doi:10.1046/j.1528-1157.2001.10100.x
6. Meletti S, Lucchi C, Monti G, et al. Decreased allopregnanolone levels in cerebrospinal fluid obtained during status epilepticus. *Epilepsia*. 2017;58(2): e16-e20. doi:10.1111/epi.13625

7. Lucchi C, Costa AM, Rustichelli C, Biagini G. Allopregnanolone and pregnanolone are reduced in the hippocampus of epileptic rats, but only allopregnanolone correlates with seizure frequency. *Neuroendocrinology*. 2021;111(6):536-541. doi:10.1059/000509093
8. Gol M, Costa AM, Biagini G, Lucchi C. Seizure progression is slowed by enhancing neurosteroid availability in the brain of epileptic rats. *Epilepsia*. 2024;65(3):e41-e46. doi:10.1111/epi.17887
9. Lucchi C, Costa AM, Senn L, Messina S, Rustichelli C, Biagini G. Augmentation of endogenous neurosteroid synthesis alters experimental status epilepticus dynamics. *Epilepsia*. 2020;61(9):e129-134. doi:10.1111/epi.16654
10. Costa AM, Gol M, Lucchi C, Biagini G. Antiepileptogenic effects of trilostane in the kainic acid model of temporal lobe epilepsy. *Epilepsia*. 2023;64(5):1376-1389. doi:10.111/epi.17561
11. Van Nieuwenhuyse B, Raedt R, Sprengers M, et al. The systemic kainic acid model of temporal lobe epilepsy: long-term EEG monitoring. *Brain Res*. 2015;1627:1-11. doi:10.1016/j.brainres.2015.08.016
12. Levesque M, Biagini G, Avoli M. Neurosteroids and focal epileptic disorders. *Int J Mol Sci*. 2020;21(24):9391. doi:10.3390/ijms21249391