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Pregnant Women with More Seizures Have Lower Allopregnanolone Concentrations

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Abstract

Neuroactive steroids have rapid, nongenomic effects on neuronal excitability. The effects in humans are less clear. We compared seizure control and concentrations of neuroactive steroids, known to influence neuroexcitability in animal studies, in pregnant women. Participants were prospectively followed throughout pregnancy with seizure-medication diaries and blood samples, assayed for steroid concentrations with gas chromatography-mass spectrometry. Baseline seizure frequency was calculated for the preconception year, and it was determined if seizure frequency was increased in each trimester. The Wilcoxon rank-sum test was used to compare neuroactive steroid concentrations in between the group with increased frequency to the group without, as calculated for the respective trimester, with the Holm-Bonferroni method to correct for multiple comparisons.

Among eighty-three pregnancies included, twenty-eight had increased seizure frequency during at least one trimester (15, 18 and 10, respectively) compared to preconception seizure frequency. Allopregnanolone concentrations were lower in the 3rd trimester (p< 0.001), with a similar trend in the 1st (p=0.08), for pregnancies with increased compared to those with stable seizure frequency.
frequency. Other neuroactive steroid concentrations were similar. Our findings suggest that lower allopregnanolone concentrations are associated with increased seizure frequency during pregnancy. Validation of these finding in a larger cohort has potential important clinical applications.

**Keywords**
neuroactive steroids; epilepsy; pregnancy; progesterone; estradiol

**Introduction**

Neuroactive steroids (NAS) are sex steroid hormones and their metabolites that exert rapid nongenomic effects on neuronal excitability. Allopregnanolone (ALLO), 5α-tetrahydrodeoxycorticosterone (THDOC), and progesterone (PROG) demonstrate anticonvulsant effects and 17β-estradiol (EST) demonstrates proconvulsant effects in animal studies. Sex hormones may have additional delayed genomic effects on neuronal excitability. Studies in humans are limited and have not been conclusive. During pregnancy the maternal-fetal-placental unit becomes the main producer of many sex steroid hormones, leading to dramatic increases in circulating NAS blood concentrations far above the non-pregnant state. The effects of circulating NAS on seizure control during pregnancy have not been studied.

With the hypothesis that seizures are likely affected by circulating NAS during pregnancy in women with epilepsy, we aimed to compare concentrations of ALLO, PROG, EST, and THDOC during pregnancy between women with stable seizure frequency and those with increased seizure frequency, as compared to their individualized non-pregnant baseline seizure frequency.

**Methods**

**Study population**

Women with epilepsy planning to conceive or pregnant at <16-weeks gestational age were screened for inclusion in a prospective observational NIH-funded study at the Emory Clinic between 2003 and 2007. The primary aims of the original study were focused on pharmacokinetic modeling of anti-seizure medications (ASMs) during pregnancy and effects on seizure control. Exclusion criteria were age <16 years, uncontrolled thyroid disease, severe anemia, ethanol or illicit drug use, renal or hepatic dysfunction, active suicidal ideation, progressive cerebral disease, known poor ASM adherence, and inability to keep a seizure calendar. The current analyses were restricted to women who were compliant with medications and follow up, had detailed seizure information and had at least two NAS measurements.

**Standard Protocol Approvals, Registrations, and Patient Consents**

The Institutional Review Board of Emory University School of Medicine approved the study. Written informed consent was obtained prior to any study procedures.
**Study design**

Study visits occurred every 1 to 3 months during pregnancy and the first postpartum year. Daily calendars were kept for concomitant medications, ASM type and doses, any missed doses, and the number and types of seizures. At each study visit, daily seizure-medication calendars were reviewed, and maternal blood was collected. The study protocol did not dictate clinical care decisions. The primary management of seizures and ASM dosing was assumed by the project principal investigator (P.B.P.). Recommendations to adjust ASM doses were based on the individual’s seizure types, epilepsy syndrome, seizure frequency, history of medication-related side effects, and pre-conception baseline ASM concentrations. The patients in the study were treated with seven different ASMs, four enzyme-inducing (carbamazepine, topiramate, phenytoin, oxcarbazepine) and three non-enzyme inducing (lamotrigine, levetiracetam, valproate).

**Human Plasma Sample Processing and NAS Quantification**

Maternal venous blood was collected at research visits between January 2003 and October 2007 and centrifuged at 2750 rpm at 3°C for 10 minutes. Serum and plasma were aliquoted into polypropylene tubes and stored at −80°C. For the samples with remaining plasma aliquots after ASM assays, they were thawed and processed for NAS extraction, derivatization and quantification by gas chromatography-mass spectrometry (GC-MS), at Tufts during May-August 2010, as previously described\(^\text{10}\).

**Data analysis**

The pregnancies included in this analysis had detailed seizure history recorded retrospectively from one year prior to conception to enrollment, and prospectively from enrollment until delivery. Postpartum data was not included in this analysis. Seizure frequency was calculated as the total number of seizures (all types) divided by number of gestational weeks over the specified time-periods: first trimester (0–14 weeks), second trimester (15–28 weeks) and third trimester (29 weeks – delivery). Seizure frequency in each trimester was compared to pre-conception baseline, and coded as 1 if “increased”, or as 0 otherwise (“stable”), thus the same woman may have been included in different groups at different time points.

The number of samples available for analysis for each participant varied for several reasons (enrollment window was preconception to 16 weeks GA, pregnancy loss). A Pearson’s chi-squared test was performed to examine the relation between seizure control (increased at any trimester or stable over the course of the entire pregnancy) and age, sex, seizure type or ASM regimen. The Wilcoxon rank-sum test was used to compare NAS concentrations by trimester between seizure control status types (increased or stable), where seizure controls status was determined at each trimester, not overall. All testing was two-tailed and p-values less than 0.05 were considered statistically significant except for the NAS concentrations, where a Holm-Bonferroni correction was applied.

A sensitivity/specificity analysis for ALLO concentration was performed for the last third ALLO measurements available within a patient and worsening seizure status (yes/no). The Youden’s index (cutoff associated with the maximum combined sensitivity and specificity)
was used to determine ALLO threshold concentration associated with an increased risk of seizure worsening.

**Results**

**Clinical characteristics and seizure frequency**

Eighty-three pregnancies (75 patients with one pregnancy and 4 patients with 2 pregnancies), contributing 287 samples, met inclusion criteria for this analysis (Table 1). Another 16 pregnancies were not included in these analyses due to lack of adequate seizure information or <2 NAS measurements. Range of age at delivery was 16–38 years (average 29; mean 30). The majority of pregnancies (84.3%) were exposed to an ASM monotherapy: 42 lamotrigine, 8 carbamazepine, 5 topiramate, 4 levetiracetam, 4 phenytoin, 3 oxcarbazepine, 4 valproate. Thirteen pregnancies were exposed to ASM polytherapy. Twenty-nine (34.9%) pregnancies were on an ASM regimen that included an enzyme-inducing ASM.

Fifty-five pregnancies had the same or improved seizure frequency during pregnancy as prior to conception. Twenty-eight (33.7%) pregnancies, had seizure worsening during at least one trimester: 15 (18.1%), 18 (21.7%), 10 (12.0%), respectively. None of the clinical characteristics reported in Table 1 were significantly different (all p > 0.05) between the group with increased versus stable seizure frequency over the course of pregnancy.

**The relation between NAS concentrations and seizure frequency**

For the pregnancies with increased seizure frequency in the third trimester, significantly lower ALLO concentrations occurred during the third trimester compared to the ALLO concentrations in pregnancies without seizure worsening (p < 0.001; statistically significant after Holm-Bonferroni correction). A similar, but non-significant trend was also seen in the first trimester (p=0.08) (Figure 1A). No statistically significant changes were noted for PROG, although a trend towards a reverse correlation with seizure control was noted in the third trimester, with higher concentrations for women with increased seizure frequency (p=0.07) (Figure 1B). No statistically significant differences were found for EST or THDOC (Figure 1D).

A sensitivity/specificity analysis using the last third trimester ALLO concentration available for each patient as a predictor for seizure worsening was performed and revealed that an ALLO concentration of 11.22 ng/mL had the maximum combined sensitivity and specificity. Note that ALLO concentrations lower than this threshold are indicative of a higher risk of seizures (Table 2).

**Discussion**

Despite a modest number of participants, our study is the first to explore the circulating NAS concentrations in pregnant women with epilepsy and to report an association between lower ALLO concentrations and worse seizure control during pregnancy. Our findings support significant ALLO differences during the 3rd trimester and reveal a trend in the same direction during the 1st trimester, consistent with animal studies that demonstrate that
ALLO is a potent positive modulator of the GABA-A receptor, with anti-seizure effects. The reverse correlation in the 3rd trimester with PROG concentrations reflects perhaps a higher rate of PROG conversion to its NAS metabolites in these women and is aligned with more recent studies suggesting that PROG may have opposite proconvulsant effects through its genomic actions.

The lack of findings with estradiol, thought to be proconvulsant, may be due to the low number or participants and samples or that its effects on seizure control in pregnant women with epilepsy are not as potent as the other NAS. A trend for higher EST concentrations in the second trimester for women with increased seizures frequency is noted.

We included pregnancies exposed to enzyme-inducing ASMs which may have altered the NAS concentrations. We ran a sensitivity analysis on the pregnancies exposed to non-enzyme inducing ASMs and, despite even lower numbers, the same trend for lower ALLO concentrations for women with increased seizure frequency during the first and third trimester was maintained. The women in this study have been followed with therapeutic drug monitoring and medication doses have been adjusted to compensate for pregnancy-related clearance changes, thus we do not think this was a significant contributor to seizure exacerbation, but clearance differences between patients may have contributed. Nonpregnant baseline NAS concentration was calculated from the preconception samples or, if not available, >4 weeks postpartum, but this information was not included in the analysis given random timing during menstrual cycle preconception and lack of information on postpartum breastfeeding status or contraceptive use which may have altered these values. Of note, our study does not include a pregnant healthy control group for NAS concentrations, nor a nonpregnant epilepsy group to assess for other factors that may play a role for seizure frequency (e.g. preconception seizure frequency). Finally, there is likely a difference in susceptibility to the actions of neuroactive steroids between women with epilepsy, and future studies allowing to gather more information on other indicators (e.g. catamenial pattern, mood changes) will be instrumental for deciphering the pathophysiology and personalizing treatment.

This small, pilot study does not allow for definitive conclusions, yet it suggests clinically relevant findings that require confirmation in studies with a larger number of participants, especially given potential important applications for improving the clinical care of pregnant women with epilepsy. We provide a third trimester ALLO threshold concentration to help providers identify women at risk of seizure worsening. In the future, if lower ALLO concentrations during pregnancy are associated with seizure worsening in a larger and independent validation cohort, then this would support pursuing new therapeutic avenues of NAS supplementation during high-risk pregnancies with poor seizure control, rather than increasing fetal exposure to larger burdens of ASMs\(^{11-14}\). Supplemental PROG is already used for the treatment of threatening or recurrent miscarriages\(^{15}\), prevention of preterm births\(^{16}\) and ALLO for postpartum depression\(^{17}\). Adjunctive PROG or ALLO may stabilize control of neuropsychiatric disease during pregnancy without having to increase ASM dosages, especially during critical and vulnerable, developmental windows.
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Conflicts of interest

Dr. V oinescu received honoraria for lectures from Brainwork.

Dr. Kurt Pennell reports grants from National Institute of Neurological Disorders and Stroke, during the conduct of the study.

Dr. Bay has no disclosures to report.

Dr. Stowe reports grants from National Institute of Health, grants from Center for Disease Control, personal fees from Harvard University - External Promotion Committee, personal fees from Sage Therapeutic - Advisory Board, other from Janssen/Johnson and Johnson, outside the submitted work.

Dr. Peng has no disclosures to report.

Dr. Frye reports grants from National Institute of Neurological Disorders and Stroke, other from How Fryed am I and Why?, LPP, during the conduct of the study.

Ms. Tang has no disclosures to report.

Dr. Page Pennell reports grants from National Institute of Neurological Disorders and Stroke, grants from National institute of Mental Health, grants from Karger Fund, during the conduct of the study; personal fees from UpToDate, Inc., Wolters Kluwer, outside the submitted work.

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References


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Figure 1.
NAS concentrations (ng/mL) for each trimester (TM) during pregnancy, separated by cohorts of women with increased seizure frequency (red) compared with stable seizure frequency (blue). A. Allopregnanolone (ALLO); B. progesterone (PROG); C. estradiol (EST); D. 5α-tetrahydrodeoxycorticosterone (THDOC).
Table 1.
Clinical characteristics of pregnancies included in the study.

<table>
<thead>
<tr>
<th>Clinical Characteristics of Pregnancies, n (%)</th>
<th>Seizure frequency</th>
<th>Total</th>
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<tbody>
<tr>
<td>Seizure Frequency</td>
<td>Stable</td>
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<tr>
<td>Worsening during this interval, n (%)</td>
<td>Entire Pregnancy</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Trimester 2</td>
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<td>Trimester 3</td>
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<td>Race, n (%)</td>
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<tr>
<td>Seizure type, n (%)</td>
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<td>Generalized</td>
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<td>ASM Regimen, n (%)</td>
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<td>Polyttherapy</td>
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<td>Number of samples, n</td>
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<td></td>
<td>Trimester 3</td>
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Table 2.
Allopregnanolone concentration thresholds predicting seizure worsening during the third trimester with their corresponding sensitivity and specificity. (*Note that decreasing ALLO values are associated with a higher risk of worsening seizure)

<table>
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<th>AP Threshold (ng/mL)</th>
<th>Specificity</th>
<th>Sensitivity</th>
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<td>33.38</td>
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<td>30.73</td>
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<td>20.93</td>
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<td>18.76</td>
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<td>14.29</td>
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<td>10.79</td>
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