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# 11β-Hydroxylase inhibitors protect against seizures in mice by increasing endogenous neurosteroid synthesis

Rafal M. Kaminski [a,1], Michael A. Rogawski [a,b,*]

[a] Epilepsy Research Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA
[b] Department of Neurology and Center for Neuroscience, University of California, Davis, Sacramento, CA, USA

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ABSTRACT

Steroid 11β-hydroxylase (CYP11B1; EC 1.14.15.4) is a mitochondrial enzyme located in the zona fasciculata of the adrenal cortex and also in the brain that mediates the conversion of 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone. Inhibitors of CYP11B1, such as metyrapone and etomidate, reduce glucocorticoid synthesis and raise levels of DOC providing greater availability for metabolic conversion to the $GABA_A$ receptor modulating neurosteroid allotetrahydrodeoxycorticosterone (THDOC). Because THDOC is a potent anticonvulsant, it is plausible that CYP11B1 inhibitors could protect against seizures. Here we demonstrate that metyrapone affords dose-dependent protection against 6-Hz seizures 30 min after injection ($ED_{50}$, 191 mg/kg), but is markedly more potent at 6 h ($ED_{50}$, 30 mg/kg). Similarly, etomidate is also protective at 30 min and 6 h ($ED_{50}$ values, 4.5 and 1.7 mg/kg). Finasteride, an inhibitor of neurosteroid synthesis, attenuated the anticonvulsant effects of both CYP11B1 inhibitors at 6 h, but not 30 min following their injection. Plasma THDOC levels measured by liquid chromatography–mass spectrometry were markedly increased 6 h after injection of both CYP11B1 inhibitors and this increase was attenuated by finasteride pretreatment. We conclude that inhibition of CYP11B1 causes delayed seizure protection due to slow build-up of neurosteroids. Early seizure protection is independent of neurosteroids.
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## 1. Introduction

Steroid 11β-hydroxylase (CYP11B1; EC 1.14.15.4) is a mitochondrial enzyme located in the zona fasciculata of the adrenal cortex and also in the brain that converts 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone (Bureik et al., 2002). CYP11B1 inhibition leads to a reduction in cortisol levels, which activates the hypothalamic–pituitary–adrenal axis (HPA) via a positive feedback mechanism (Allolio et al., 1985; Sillence and Rodway, 1987; Schulte et al., 1990). The resulting ACTH surge drives the adrenal glands leading to the build-up of upstream precursors, which are then shunted to alternative metabolic pathways. Specifically, levels of DOC are increased providing greater availability for metabolic conversion to the $GABA_A$ receptor modulating neurosteroid allotetrahydrodeoxycorticosterone (THDOC) (Fig. 1). There may also be increases in the levels of the DOC precursor progesterone, which can be converted to the other prototypic $GABA_A$ receptor modulating neurosteroid allopregnanolone. There may even be increases in androgens that are metabolized through testosterone to 5α-androstanediol, androsterone and etiocholanolone, all of which positively modulate $GABA_A$ receptors (Kaminski et al., 2005; Reddy and Jian, 2010). Because all of these neurosteroids have anticonvulsant properties (Kokate et al., 1994; Reddy and Rogawski, 2002; Kaminski et al., 2005), it is plausible that CYP11B1 inhibitors could protect against seizures. We investigated this possibility using two inhibitors of CYP11B1, metyrapone and etomidate (Carballeira et al., 1976; Dörr et al., 1984; De Coster et al., 1985; Lambert et al., 1986; Weber et al., 1993). Metyrapone is used clinically in the treatment of Cushing's syndrome (hypercorticism). Etomidate is a parenteral anesthetic agent that was discovered shortly after its introduction to induce hypoadrenalism. While this action is generally considered a liability, etomidate has been used to correct hypercortisolemia in seriously ill patients. The protective actions of metyrapone and etomidate were assessed with the 6-Hz seizure model in mice that has been previously shown to be sensitive to neurosteroids (Kaminski et al., 2004). To confirm that treatment with the CYP11B1 inhibitors is associated with increased neurosteroid synthesis we measured plasma levels of THDOC by liquid chromatography–mass spectrometry (LC–MS). We then

*Abbreviation:* THDOC, allotetrahydrodeoxycorticosterone.

* Corresponding author. Department of Neurology, University of California, Davis, 4860 Y Street, Suite 3700, Sacramento, CA 95817, USA.
*E-mail address:* rogawski@ucdavis.edu (M.A. Rogawski).
[1] Present address: UCB Pharma S.A., Chemin du Foriest, R9, B-1420 Braine-l'Alleud, Belgium.

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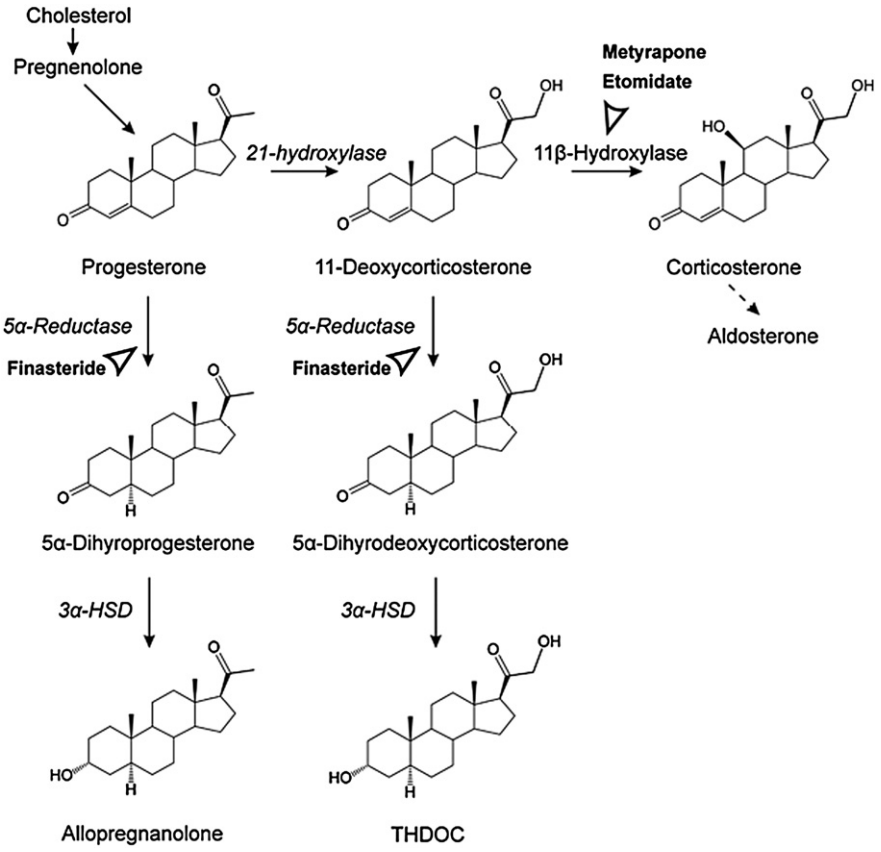


**Fig. 1.** Adrenal steroid biosynthetic pathways illustrating shunting toward the production of the neurosteroids allopregnanolone and THDOC when 11β-hydroxylase is inhibited by metyrapone and etomidate. Finasteride inhibits 5α-reductase isoenzymes, reducing neurosteroid production. 3α-HSD, 3α-hydroxysteroid oxidoreductase isoenzymes. Open arrowheads denote enzyme inhibition.

used the neurosteroid synthesis inhibitor, finasteride (Kokate et al., 1999), to assess whether enhanced neurosteroidogenesis is causally related to the seizure protection conferred by the two CYP11B1 inhibitors.

## 2. Material and methods

### 2.1. Animals

Male NIH Swiss mice (25–30 g) were housed three per cage in a vivarium under controlled laboratory conditions (temperature, 22–26 °C; humidity, 40–50%) with an artificial 12-h light/dark cycle and free access to food and water. Animals were allowed to acclimate to the vivarium conditions for at least 5 days. The experiments were performed during the light phase of the light/dark cycle after at least a 0.5 h period of acclimation to the experimental room. Animals were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and studies were performed under protocols approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke (NINDS) in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academy Press, Washington, DC; http://www.nap.edu/readingroom/books/labrats/).

### 2.2. Drug administration and neurosteroid measurement

Solutions of etomidate (Sigma–Aldrich, St. Louis, MO) and finasteride (Steraloids, Newport, RI) were made fresh daily in 40% hydroxypropyl-β-cyclodextrin (Trappsol; Cyclodextrin Technologies Development, High Springs, FL) in sterile saline. Further dilutions were made using sterile saline. Metyrapone (Sigma–Aldrich) was dissolved in sterile saline. All drug solutions were administered intraperitoneally in a volume equaling 10 ml/kg at different times before seizure testing or blood collection. The mice were decapitated under brief carbon dioxide narcosis. Trunk blood was collected into tubes containing anticoagulant (EDTA sodium) and centrifuged at 5000 rpm for 15 min at 4 °C. Subsequently blood plasma was transferred to Eppendorf tubes and stored at −70 °C until THDOC measurements were made by LC–MS as previously described (Reddy and Rogawski, 2002).

### 2.3. 6-Hz seizure test

Anticonvulsant activity at different time points after drug treatment was assessed using a low-frequency electrical stimulation seizure test (Barton et al., 2001) applied according to the protocol of Kaminski et al. (2004). In brief, 3-s corneal stimulation (200-μs duration, 32-mA monopolar rectangular pulses at 6 Hz) was delivered by a constant-current device (ECT Unit 5780; Ugo Basile, Comerio, Italy). Ocular anesthetic (0.5% tetracaine) was applied to the corneas 15 min before stimulation. Immediately before stimulation, the corneal electrodes were wetted with saline to provide optimal electrical contact. After the stimulation, the animals exhibited a "stunned" posture associated with rearing and automatic movements that lasted from 60 to 120 s in untreated animals. An animal was considered to be protected from seizures if it resumed its normal exploratory behavior within 10 s of stimulation.

### 2.4. Data analysis

To construct dose–response curves, metyrapone and etomidate were tested at several doses spanning the dose estimated to produce 50% protection ($ED_{50}$), which was determined together with its 95% confidence interval by non-linear curve fitting with GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). Statistical comparisons between $ED_{50}$ values were made with the same software. Statistical comparisons in the time-course experiments assessing protection against 6-Hz seizures following treatment with the CYP11B1 inhibitors were made with the Fisher's exact test. Mean plasma levels of THDOC were compared with the Student's *t*-test. At least 6–8 mice were included in each experimental group.

## 3. Results

Administration of metyrapone (100 mg/kg) resulted in the slow development of seizure protection in the 6-Hz test, reaching maximum effect at 6 h post-treatment (Fig. 2A). Seizure protection declined during the subsequent 18 h of monitoring. In dose–response experiments, treatment with metyrapone at doses of