Published in final edited form as:

Epilepsy Res. 2010 May ; 89(2-3): 254–260. doi:10.1016/j.epilepsyres.2010.01.009.

Ganaxolone Suppression of Behavioral and Electrographic Seizures in the Mouse Amygdala Kindling Model

Doodipala S. Reddy^{1,*} and Michael A. Rogawski²

¹ Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M System Health Science Center, College Station, TX 77843

² Department of Neurology, School of Medicine and Center for Neuroscience, University of California, Davis, Sacramento, CA 95817, USA

Summary

Ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), a synthetic analog of the endogenous neurosteroid allopregnanolone and a positive allosteric modulator of GABA_A receptors, may represent a new treatment approach for epilepsy. Here we demonstrate that pretreatment with ganaxolone (1.25–20 mg/kg, s.c.) causes a dose-dependent suppression of behavioral and electrographic seizures in fully amygdala kindled female mice, with nearly complete seizure protection at the highest dose tested. The ED₅₀ for suppression of behavioral seizures was 6.6 mg/kg. The seizure suppression produced by ganaxolone was comparable to that of clonazepam (ED₅₀, 0.1 mg/kg, s.c.). To the extent that amygdala kindling represents a model of mesial temporal lobe epilepsy, this study supports the utility of ganaxolone in the treatment of patients with temporal lobe seizures.

Keywords

ganaxolone; neurosteroid; clonazepam; GABA_A receptor; amygdala kindling; epilepsy; seizure; female mice

Introduction

Ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), the 3 β -methyl analog of the progesterone metabolite allopregnanolone, is the first neurosteroid related agent to be evaluated in man for the drug treatment of epilepsy (Nohria and Giller, 2007). According to a widely held definition, neurosteroids are endogenous steroids that directly modulate neural excitability through non-genomic actions. The most studied and best accepted molecular target of neurosteroids is GABA_A receptors (Belelli et al., 2005; Reddy, 2009). Ganaxolone, like allopregnanolone, acts as a powerful positive allosteric modulator of GABA_A receptors, resulting in enhanced neural inhibition. As is the case for other agents that enhance GABA_A

*Address for correspondence: D. Samba Reddy, Ph.D., R.Ph., Associate Professor, Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M Health Science Center, 228 Reynolds Medical Building, College Station, TX 77843-1114, Tel: 979-862-2852; Fax: 979-845-0790, red dy@medicine.tamhsc.edu.

Disclosure

M.A.R. is a scientific founder of and has served as a consultant to Marinus Pharmaceuticals, Inc., Branford, CT.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

receptor mediated inhibition, ganaxolone exhibits protective activity in diverse animal seizure models (Rogawski and Reddy, 2004; Liptáková et al., 2000; Gasior et al., 2000). Moreover, in phase 2 clinical trials, ganaxolone treatment has been found to reduce the frequency of partial seizures in adult patients (Laxer et al., 2000; Marinus Pharmaceuticals, unpublished). Neurosteroid-related anticonvulsants like ganaxolone have unique properties that distinguish them from other anticonvulsants that act on brain GABA systems. For example, GABA_A receptor modulating neurosteroids preferentially act on extrasynaptic (δ -subunit-containing) GABA_A receptors (Glykys and Mody, 2007) and tolerance to the anticonvulsant effects of neurosteroids generally does not occur when they are administered systemically (Reddy and Rogawski, 2000a). In comparison with endogenous 3 α -hydroxy-pregnane neurosteroids such as allopregnanolone, ganaxolone has the advantage that it cannot be converted by 3 α -hydroxysteroid oxidoreductase isoenzymes to the hormonally active 3-keto form (Rupprecht et al., 1993), and hence it is believed that patients taking ganaxolone would not be at risk of hormonal side effects (Monaghan et al., 1997; Reddy and Woodward, 2004).

Amygdala kindling has been proposed as a model of human temporal lobe epilepsy (Albright and Burnham, 1979). To elicit the kindled state, a mild focal electrical stimulus is applied unilaterally to the amygdala on a daily basis causing a local electrographic seizure known as the “afterdischarge.” With time, the initially behaviorally innocuous electrical stimulus elicits increasingly more robust limbic seizure-like behavioral events (Goddard et al., 1969). The kindled state is stable during the animal’s lifetime such that the kindling stimulus always elicits a full-blown behavioral and electrographic seizure. The kindling model has been widely used in the evaluation of drugs for antiseizure activity. Previous studies have indicated that neurosteroids with GABA_A receptor modulatory activity have protective effects in the amygdala kindling model (Holmes and Weber, 1984; Reddy and Rogawski, 2002; Reddy et al., 2004; Lonsdale et al., 2006; Lonsdale and Burnham, 2007). Moreover, ganaxolone itself has been shown to protect against various forms of kindled seizures in mice and rats, including corneal kindling (Carter et al., 1997) and cocaine kindling (Gasior et al., 2000; Kaminski et al., 2003). However, ganaxolone has not been previously studied in the amygdala kindling model, which is the best established kindling model and shows pharmacological differences from other kindling models used for anticonvulsant drug evaluation (Potschka and Löscher, 1999). Since ganaxolone is under clinical development, we sought to characterize its activity in the amygdala kindling model in mice. For comparison, we conducted parallel experiments with clonazepam, which has previously been shown to be highly effective against amygdala kindled seizures (Ashton and Wauquier, 1979; Albertson et al., 1980; Löscher et al., 1986; Tietz et al., 1989; Fukinaga et al., 1998). Our results indicate that, in fully kindled mice, ganaxolone produces a marked suppression of stimulation-evoked behavioral seizures and electrographic seizure activity, supporting the potential utility of ganaxolone to treat complex partial seizures.

Materials and methods

Animals

Adult female mice of C57BL6 strain weighing approximately 25–30 g were used in the study. The mice were housed in an environmentally controlled animal facility under a normal 12 h/12 h light/dark cycle and allowed free access to food and water, except during the experimental sessions. The mice were randomly assigned for drug testing and did not check for ovarian cycle stages. Female mice were used the study because of targeted indication of ganaxolone for treating women with epilepsy. All procedures were performed in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the Institutional Animal Care and Use Committee.

Electrode implantation

Electrode implantation and stimulation procedures for mouse amygdala kindling were performed as described previously (Rogawski et al., 2001; Reddy and Rogawski, 2002). Briefly, mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). A twisted bipolar stainless steel wire electrode (model MS303/1; Plastic One, Roanoke, VA) was stereotactically implanted in the right amygdala complex (1.3 mm posterior and 3.0 mm lateral to bregma, and 4.6 mm below the dorsal surface of the skull) (Franklin and Paxinos, 1997) and anchored with dental acrylic to three jeweler's screws placed in the skull. A period of 7 to 10 days was allowed for recovery.

Kindling procedure

The stimulation paradigm consisted of 1 ms-duration, bipolar, square current pulses delivered at 60 Hz for 1 s using a kindling stimulator (A-M Systems, Sequim, WA). The afterdischarge threshold was determined by stimulating at 5 min intervals beginning with an intensity of 75 μ A and increasing in steps of 50 μ A until an afterdischarge of at least 5 s was obtained. Stimulation on subsequent days used a stimulation intensity 125% of the threshold value. Seizure activity following each stimulation was rated according to the criterion of Racine (1972) as modified for the mouse: stage 0, no response or behavior arrest; stage 1, chewing or head nodding; stage 2, chewing and head nodding; stage 3, forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling. The afterdischarge was recorded from the amygdala electrode with a Grass CP511 AC electroencephalogram preamplifier (Astro-Med, West Warwick, RI) and stored in digital form using Axoscope 8.1 (Axon Instruments, Foster City, CA). Afterdischarge duration was the total duration of amygdala electrographic spike activity (amplitude $> 2 \times$ baseline) occurring in a rhythmic pattern at a frequency > 1 Hz. The afterdischarge often exhibited two phases of waxing and waning amplitude; when this occurred, the afterdischarge duration was taken as the time to the end of the second phase. The day of afterdischarge threshold determination was considered day 1 of kindling. Kindling stimulation was delivered daily until stage 5 seizures were elicited on three consecutive days. Stimulation was continued on a five-day per week schedule each afternoon. Mice were used for drug testing when they consistently exhibited stage 5 seizures with stimulation, which is considered the "fully kindled" state.

Kindling seizure studies

To examine the ability of the test drugs (ganaxolone or clonazepam) to suppress the expression of kindled seizures, the kindled mice underwent a 3-day test protocol. On day 1, they were verified to exhibit a stimulation-induced stage 5 seizures. On day 2, a subcutaneous ganaxolone injection was administered 15 min prior to stimulation. On day 3, the animals were stimulated again without drug pretreatment. During each test session, the behavioral seizure score and the afterdischarge duration (recorded from the implanted amygdala stimulating electrode) were noted. The seizure scores and afterdischarge durations on days 1 and 3 were averaged and taken as the control values. At the end of the study, mice were anesthetized and perfused transcardially with paraformaldehyde for Nissl staining to verify the electrode placement.

Data analysis

Group data are expressed as the mean $\pm$ standard error of the mean (S.E.M.); differences in seizure stage between groups were compared with the nonparametric Kruskal-Wallis test followed by the Mann-Whitney *U*-test. Comparison of means of the afterdischarge duration between groups was made with one-way analysis of variance, followed by unpaired two-tailed Student's *t*-test. Comparison of the mean percentage inhibition of seizure stage and afterdischarge duration in fully kindled animals was made by Wilcoxon signed ranks test and paired two-tailed Student's *t*-test, respectively. ED₅₀ (estimated dose resulting in 50%

inhibition) values were determined by non-linear curve fitting using the Levenberg-Marquardt algorithm to a logistic equation where the maximum inhibition was assumed to be 100%. In all statistical tests, the criterion for statistical significance was $p < 0.05$.

Drugs

Stock solutions of ganaxolone for injection were made in 30% hydroxypropyl- β -cyclodextrin in water, and additional dilutions were made using normal saline. By itself, β -cyclodextrin at concentrations as high as 50% failed to affect kindled seizures. Drug solutions were administered s.c. or i.p. in a volume equaling 1% of the animal's body weight. Ganaxolone and clonazepam were purchased from Sigma (St. Louis, MO).

Results

Amygdala kindling development

The initial kindling stimulation most commonly evoked no behavioral response; occasionally there was behavioral arrest or facial movement. The overall average value of the initial afterdischarge threshold was $373 \pm 40 \mu\text{A}$ ($n=8$). Daily kindling stimulation was associated with an orderly progression to increasing behavioral seizure stage (Fig. 1A). The early phase of kindling was associated with a gradual prolongation in the afterdischarge duration after which the duration remained in the range of 45 to 80 s (Fig. 1B). Mice reached the fully kindled state in which they consistently exhibited stage 5 seizures after about 14 stimulations.

Ganaxolone protection against amygdala-kindled seizures

To evaluate the activity of ganaxolone in protecting against amygdala kindled seizures, fully kindled mice were treated with various doses of ganaxolone 15 min prior to stimulation. As shown in Fig. 2, ganaxolone produced a dose-dependent suppression of behavioral seizure activity (Fig. 2A) and afterdischarge duration (Fig. 2B) with significant effects on both at 5, 10 and 20 mg/kg. The percentage inhibition at the various doses is shown in Fig. 4A. At the highest dose tested, behavioral seizures were nearly completely suppressed. The estimated ED_{50} value for suppression of seizure stage and afterdischarge duration is $6.6 \pm 1.1 \text{ mg/kg}$ and $11 \pm 1.6 \text{ mg/kg}$, respectively. Sample recordings of afterdischarges from one animal are shown in Fig. 3. Ganaxolone pretreatment markedly reduced the duration of the afterdischarge duration in a dose-dependent fashion. In addition, the overall amplitude was reduced by about 50% after 5 and 20 mg/kg ganaxolone. On the day after ganaxolone treatment, all mice exhibited full-blown stage 5 seizures with afterdischarge duration ($51.0 \pm 2.6 \text{ s}$) not significantly different from the control duration ($55.5 \pm 4.0 \text{ s}$), indicating that ganaxolone treatment suppresses the expression of kindled seizures but does not alter the kindled state.

Clonazepam protection against amygdala-kindled seizures

For comparison, experiments were conducted in the same mice with clonazepam after a two week washout period. As shown in Fig. 2, clonazepam treatment produced a dose-dependent suppression of the behavioral seizure score (Fig. 2C) and afterdischarge duration (Fig. 2D). The percentage inhibition at the various doses is shown in Fig. 4B. The estimated ED_{50} values for suppression of seizure stage and afterdischarge duration is 0.10 ± 0.02 and $0.10 \pm 0.03 \text{ mg/kg}$, respectively.

Discussion

In this study, the synthetic neurosteroid analog ganaxolone was found to have powerful antiseizure activity in the mouse amygdala kindling model of epilepsy. Animals were fully kindled and then tested with a supramaximal stimulus that was 125% of their individual

afterdischarge threshold. Pretreatment with ganaxolone caused a dose-dependent reduction in behavioral seizure score and a corresponding suppression of the duration of electrographic discharges. The behavioral and electrographic features of kindled seizures have similarities to human limbic seizures and the neuropathology is comparable to that observed in human mesial temporal lobe epilepsy (Sutula, 1990). Therefore, kindling is considered to be a model of human complex partial epilepsy and it is often used in antiepileptic drug development (Albertson et al., 1980). Our results support the utility of ganaxolone in the treatment of complex partial seizures.

Ganaxolone has antiseizure potency in the mouse amygdala kindling model that is similar to its potency in other seizure models in which it is highly active. Table 1 compares the potency of ganaxolone against amygdala kindled seizures with its potencies in other mouse seizure models. As is the case for neurosteroids and other agents that act as positive modulators of GABA_A receptors, ganaxolone is effective against seizures induced by chemoconvulsants that antagonize GABA_A receptors including pentylenetetrazol, bicuculline, *t*-butylbicycloorthobenzoate and flurothyl (Reddy and Woodward, 2004; Krasowski, 2000). It is also active in the 6 Hz electroshock model, which has been shown to be highly sensitive to agents that positively modulate GABA_A receptors (Kaminski et al., 2004). In addition, ganaxolone exhibits activity against cocaine kindled seizures (Gasior et al., 2000; Kaminski et al., 2003). At higher doses, ganaxolone protects mice against maximal electroshock (MES)-induced seizures. In general, GABA_A receptor positive modulators, such as benzodiazepines and neurosteroids, have weak activity in the MES test (Kokate et al., 1994).

Electrophysiological studies in *Xenopus* oocytes expressing recombinant human GABA_A receptors demonstrated that ganaxolone is a potent positive allosteric modulator of GABA_A receptor function (Carter et al., 1997). The steroid is not known to interact with other molecular targets. Therefore, it is likely that the protective activity against kindled-seizures relates to the actions on GABA_A receptors. The estimated plasma concentrations of ganaxolone at doses that protect in the kindling model (~3 μ M at ED₅₀, 6.6 mg/kg; Reddy and Rogawski, 2000a; Reddy et al., 2004) exceed the range of concentrations that modulate GABA_A receptors in *in vitro* preparations (EC₅₀, 94–213 nM; Carter et al., 1997). It seems likely that brain penetration is limiting so that target concentrations do not fully reflect the plasma concentrations. However, it is noteworthy that at relatively high (micromolar) concentrations, neurosteroids including ganaxolone directly activate GABA_A receptor channels in the absence of GABA (Kokate et al., 1994; Carter et al., 1997; Reddy and Rogawski, 2002). Such a direct agonist action could contribute to the protective activity of ganaxolone in the kindling model.

Many previous studies of ganaxolone have utilized male animals to avoid the potentially confounding influence of ovarian hormone fluctuations associated with the estrous cycle that occurs in female rodents of reproductive age. In the present study, we utilized female mice so as to obtain information of utility in assessing the potential of ganaxolone to treat women with epilepsy. Our results demonstrate that the drug is as likely to be efficacious in women as in men. Changes in seizure susceptibility are not evident among phases of the estrous cycle in fully kindled animals (Wahnschaffe and Löscher, 1992). Nevertheless, had we timed our testing to the estrous cycle, it is possible that we might have detected differences in ganaxolone sensitivity between the estrous cycle stages. In addition, since we restricted our experiments to females, our results are not strictly generalizable to males although we do note that the potency of ganaxolone obtained in the present study is similar to that obtained in male mice in other models (Table 1).

To provide a validated positive control, in the present study we conducted a parallel series of experiments with clonazepam, which is well recognized to protect against amygdala kindled seizures (Ashton and Wauquier, 1979; Albertson et al., 1980; Löscher et al., 1986; Tietz et al.,

1989; Fukinaga et al., 1998). As expected, pretreatment with clonazepam caused a dose-dependent inhibition of seizure stage and duration that was similar to that produced by ganaxolone, except that clonazepam was substantially more potent on a molar basis. The lower potency of ganaxolone is consistent with the relatively low binding affinity of neurosteroids for GABA_A receptors (Chisari et al., 2009) but also likely relates to its restricted bioavailability. It is noteworthy that antiepileptic drugs for the most part have low binding affinities for their molecular targets and that the systemic potency of ganaxolone is typical of the potencies of common antiepileptic drugs. Although benzodiazepines such as clonazepam are effective antiseizure drugs, their clinical usefulness in the chronic treatment of epilepsy is limited because tolerance develops to their protective effects with long-term therapy. In a previous study comparing ganaxolone and the benzodiazepine diazepam in a repeated-dosing paradigm, we observed that tolerance for protection against pentylenetetrazol-induced seizures occurs with diazepam but not ganaxolone (Reddy and Rogawski, 2000a). Moreover, there was no evidence of tolerance in a recent clinical trial of ganaxolone as add-on therapy for adult partial seizures (Marinus Pharmaceuticals, unpublished). Therefore, lack of tolerance may confer ganaxolone with a substantial advantage in comparison with benzodiazepines for the chronic treatment of epilepsy. Ganaxolone also appears to have a favorable safety profile and there is no evidence that it is teratogenic or causes major systemic toxicity with chronic administration, although it does cause dose-related sedation, consistent with its action on GABA_A receptors (Reddy, 2003).

In conclusion, our results demonstrate that ganaxolone has powerful antiseizure effects in the amygdala kindling model in mice, supporting the utility of ganaxolone in the treatment of temporal lobe epilepsy. The observation that ganaxolone is effective in female animals highlights its application to address the unique challenges faced in treating women with epilepsy, which include drug-resistant catamenial seizure exacerbations (Reddy, 2009; Reddy and Rogawski, 2000b, 2009).

Acknowledgments

This work was supported in part by National Institutes of Health grant NS051398 (to D.S.R.).

References

- Albertson TE, Peterson SL, Stark LG. Anticonvulsant drugs and their antagonism of kindled amygdaloid seizures in rats. *Neuropharmacology* 1980;19:643–652. [PubMed: 7402452]
- Albright PS, Burnham WM. Development of a new pharmacological seizure model: effects of anticonvulsants on cortical- and amygdala-kindled seizures in the rat. *Epilepsia* 1980;21:681–689. [PubMed: 7439134]
- Ashton D, Wauquier A. Behavioral analysis of the effects of 15 anticonvulsants in the amygdaloid kindled rat. *Psychopharmacology (Berl)* 1979;65:7–13. [PubMed: 116293]
- Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA_A receptor. *Nat Rev Neurosci* 2005;6:565–575. [PubMed: 15959466]
- Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), a selective, high-affinity, steroid modulator of the γ -aminobutyric acid_A receptor. *J Pharmacol Exp Ther* 1997;280:1284–1295. [PubMed: 9067315]
- Chisari M, Eisenman LN, Krishnan K, Bandyopadhyaya AK, Wang C, Taylor A, Benz A, Covey DF, Zorumski CF, Mennerick S. The influence of neuroactive steroid lipophilicity on GABA_A receptor modulation: evidence for a low-affinity interaction. *J Neurophysiol* 2009;102:1254–1264. [PubMed: 19553485]
- Franklin, KBJ.; Paxinos, G. The mouse brain in stereotaxic coordinates. 1. Academic Press; 1997.

- Fukinaga M, Ishizawa K, Kamei C. Anticonvulsant properties of 1,4-benzodiazepine derivatives in amygdaloid-kindled seizures and their chemical structure-related anticonvulsant action. *Pharmacology* 1998;57:233–241. [PubMed: 9742288]
- Gasior M, Ungard JT, Beekman M, Carter RB, Witkin JM. Acute and chronic effects of the synthetic neuroactive steroid ganaxolone, against the convulsive and lethal effects of pentylenetetrazol in seizure-kindled mice: comparison with diazepam and valproate. *Neuropharmacology* 2000;39:1184–1196. [PubMed: 10760361]
- Glykys J, Mody I. Activation of GABA_A receptors: views from outside the synaptic cleft. *Neuron* 2007;56:763–770. [PubMed: 18054854]
- Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969;25:295–330. [PubMed: 4981856]
- Holmes GL, Weber DA. The effect of progesterone on kindling: a developmental study. *Brain Res* 1984;318:45–53. [PubMed: 6488053]
- Kaminski RM, Gasior M, Carter RB, Witkin JM. Protective efficacy of neuroactive steroids against cocaine kindled-seizures in mice. *Eur J Pharmacol* 2003;474:217–222. [PubMed: 12921865]
- Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia* 2004;45:864–867. [PubMed: 15230714]
- Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with γ -aminobutyric acid-evoked chloride current potentiation. *J Pharmacol Exp Ther* 1994;270:1223–1229. [PubMed: 7932175]
- Krasowski MD. Differential modulatory actions of the volatile convulsant flurothyl and its anesthetic isomer at inhibitory ligand-gated ion channels. *Neuropharmacology* 2000;39:1168–1183. [PubMed: 10760360]
- Laxer K, Blum D, Abou-Khalil BW, Morrell MJ, Lee DA, Data JL, Monaghan EP. Assessment of ganaxolone's anticonvulsant activity using a randomized, double-blind, presurgical trial design. Ganaxolone Presurgical Study Group. *Epilepsia* 2000;41:1187–1194. [PubMed: 10999558]
- Liptáková S, Velíšek L, Velísková J, Moshé SL. Effect of ganaxolone on flurothyl seizures in developing rats. *Epilepsia* 2000;41:788–793. [PubMed: 10897148]
- Lonsdale D, Burnham WM. The anticonvulsant effects of allopregnanolone against amygdala-kindled seizures in female rats. *Neurosci Lett* 2007;411:147–151. [PubMed: 17084970]
- Lonsdale D, Nylen K, Burnham WM. The anticonvulsant effects of progesterone and its metabolites on amygdala-kindled seizures in male rats. *Brain Res* 2006;1101:110–116. [PubMed: 16784731]
- Löscher W, Jäckel R, Czuczwar SJ. Is amygdala kindling in rats a model for drug-resistant partial epilepsy? *Exp Neurol* 1986;93:211–226. [PubMed: 3089832]
- Monaghan EP, Navalta LA, Shum L, Ashbrook DW, Lee DA. Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. *Epilepsia* 1997;38:1026–1031. [PubMed: 9579942]
- Nohria V, Giller E. Ganaxolone. *Neurotherapeutics* 2007;4:102–105. [PubMed: 17199022]
- Potschka H, Löscher W. Corneal kindling in mice: behavioral and pharmacological differences to conventional kindling. *Epilepsy Res* 1999;37:109–120. [PubMed: 10510977]
- Racine RJ. Modification of seizure activity by electrical stimulation. II Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;32:281–294. [PubMed: 4110397]
- Reddy DS. Pharmacology of endogenous neuroactive steroids. *Crit Rev Neurobiol* 2003;15:197–234. [PubMed: 15248811]
- Reddy DS. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. *Epilepsy Res* 2009;85:1–30. [PubMed: 19406620]
- Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther* 2000a;295:1241–1248. [PubMed: 11082461]
- Reddy DS, Rogawski MA. Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. *J Pharmacol Exp Ther* 2000b;294:909–915. [PubMed: 10945840]

- Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulates GABA_A receptor function and seizure susceptibility. *J Neurosci* 2002;42:3795–3805. [PubMed: 11978855]
- Reddy DS, Rogawski MA. Neurosteroid replacement therapy for catamenial epilepsy. *Neurotherapeutics* 2009;6:392–401. [PubMed: 19332335]
- Reddy DS, Castaneda DC, O'Malley BW, Rogawski MA. Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. *J Pharmacol Exp Ther* 2004;310:230–239. [PubMed: 14982969]
- Reddy DS, Woodward R. Ganaxolone: A prospective overview. *Drugs Future* 2004;29:227–242.
- Rogawski, MA.; Reddy, DS. Neurosteroids: endogenous modulators of seizure susceptibility. In: Rho, JM.; Sankar, R.; Cavazos, JE., editors. *Epilepsy: scientific foundations of clinical practice*. New York: Marcel Dekker; 2004. p. 319-355.
- Rogawski MA, Kurzman PS, Yamaguchi S, Li H. Role of AMPA and GluR5 kainate receptors in the development and expression of amygdala kindling in the mouse. *Neuropharmacology* 2001;40:28–35. [PubMed: 11077068]
- Rupprecht R, Reul JM, Trapp T, van Steensel B, Wetzel C, Damm K, Zieglgansberger W, Holsboer F. Progesterone receptor-mediated effects of neuroactive steroids. *Neuron* 1993;11:523–530. [PubMed: 8398145]
- Sutula TP. Experimental models of temporal lobe epilepsy: new insights from the study of kindling and synaptic reorganization. *Epilepsia* 1990;31(Suppl 3):S45–54. [PubMed: 2226371]
- Tietz EI, Rosenberg HC, Chiu TH. A comparison of the anticonvulsant effects of 1,4- and 1,5-benzodiazepines in the amygdala-kindled rat and their effects on motor function. *Epilepsy Res* 1989;3:31–40. [PubMed: 2917546]
- Wahnschaffe U, Löscher W. Lack of changes in seizure susceptibility during the estrous cycle in kindled rats. *Epilepsy Res* 1992;13:199–204. [PubMed: 1493782]

Figure 1.

Kindling development in adult female mice. (A) Progression of behavioral seizure stage with number of amygdala kindling stimulations. Animals were stimulated daily until stage 5 seizures were elicited on three consecutive days. Each data point represents the mean of the behavioral seizure stage of 6 to 8 animals. The overall average number of stimulations to achieve stage 5 kindling was 14.5. (B) Afterdischarge duration during the development of amygdala kindling in the same animals. Each data point represents the mean $\pm$ S.E.M. of the total afterdischarge duration obtained with each daily stimulation.

Figure 2.

Inhibition of amygdala kindled seizures by ganaxolone (A,B) and clonazepam (C,D) in female mice. Dose-response curves for behavioral seizure stage (A) and afterdischarge duration (B) with varying doses of ganaxolone (1.25–20 mg/kg, sc). Dose-response curves for behavioral seizure stage (C) and afterdischarge duration (D) with varying doses of clonazepam (0.037–0.18 mg/kg, ip). Mice that were fully kindled as described in Fig. 1 were injected subcutaneously with vehicle or ganaxolone 15 min before stimulation. Each point represents the mean $\pm$ S.E.M. of data from 6 to 8 animals. * $P < 0.05$ vs. control. A minimum of two weeks was allowed to elapse between test doses.

Figure 3.

Representative traces illustrating inhibition of afterdischarge in a fully kindled mouse by 5 and 20 mg/kg ganaxolone administered 15-min prior to stimulation. Traces show depth electroencephalographic recordings from a stimulating electrode in the right amygdala. Arrows indicate onset of the 1 s kindling stimulus, which is followed by the stimulus artifact (mostly blanked to prevent saturation of the amplifier circuitry). In each trace, a slow negative wave following stimulation was digitally subtracted. Ganaxolone was administered s.c. 15-min prior to the stimulation; control trace was obtained without drug treatment.

Figure 4.

Percent inhibition of fully kindled seizures and afterdischarge duration by ganaxolone (A) and clonazepam (B). Percent inhibition of seizure stage was calculated as $100 \times (1S/5)$, where S is the seizure stage following drug treatment. Percentage inhibition of afterdischarge was calculated as $100 \times (1-D/D_c)$, where D is the afterdischarge duration after drug treatment and D_c is the average control afterdischarge duration without any drug treatment. The overall mean control afterdischarge duration was 55.5 ± 4.0 s. Each bar represents the mean $\pm$ S.E.M. of values from 6 to 8 animals. * $P < 0.05$ vs. control.

Table 1

Antiseizure activity of ganaxolone in mouse models of epilepsy.

Seizure model	ED ₅₀ value (mg/kg)	Literature citation
Pentylenetetrazol	3.5 (2.1–5.8)	Reddy and Rogawski, 2000b
Pentylenetetrazol kindling	4.1 (2.7–6.4)	Gasior et al., 2000
Bicuculline	4.6 (3.2–6.8)	Carter et al., 1997
Flourothyl	5.0 (ND)	Liptakova et al., 2000
6 Hz	6.3 (4.0–9.8)	Kaminski et al., 2004
Amygdala kindling	6.6 (5.1–9.7)	This paper
<i>t</i> -Butylbicycloorthobenzoate	11.7 (8.8–15.7)	Carter et al., 1997
Aminophylline	11.5 (8.1–16.3)	Carter et al., 1997
Cocaine kindling	17.0 (ND)	Kaminski et al., 2003
Maximal electroshock	29.7 (25.3–34.8)	Carter et al., 1997
N-methyl-D-aspartate	> 30 (ND)	Carter et al., 1997
Strychnine	> 40 (ND)	Carter et al., 1997

Numbers in parentheses are 95% confidence intervals. ND, not determined.