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Title

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Journal

Epilepsia, 54(s6)

ISSN

0013-9580

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Publication Date

2013-09-01

DOI

10.1111/epi.12289

Peer reviewed



Published in final edited form as:

Epilepsia. 2013 September ; 54(0 6): 93–98. doi:10.1111/epi.12289.

Neuroactive Steroids for the Treatment of Status Epilepticus

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SUMMARY

Benzodiazepines are the current first-line standard-of-care treatment for status epilepticus but fail to terminate seizures in about one-third of cases. Synaptic GABA_A receptors, which mediate phasic inhibition in central circuits, are the molecular target of benzodiazepines. As status epilepticus progresses, these receptors are internalized and become functionally inactivated, conferring benzodiazepine resistance, which is believed to be a major cause of treatment failure. GABA_A receptor positive allosteric modulator neuroactive steroids, such as allopregnanolone, also potentiate synaptic GABA_A receptors, but in addition they enhance extrasynaptic GABA_A receptors that mediate tonic inhibition. Extrasynaptic GABA_A receptors are not internalized and desensitization of these receptors does not occur during continuous seizures in status epilepticus models. Here we review the broad-spectrum antiseizure activity of allopregnanolone in animal seizure models and the evidence for its activity in models of status epilepticus. We also demonstrate that allopregnanolone inhibits ongoing behavioral and electrographic seizures in a model of status epilepticus, even when there is benzodiazepine resistance. Parenteral allopregnanolone may provide an improved treatment for refractory status epilepticus.

Keywords

Refractory status epilepticus; Seizure; Allopregnanolone; Neurosteroid; Allosteric modulator

The first-line standard-of-care treatment for early and established status epilepticus is a parenteral benzodiazepine, such as lorazepam or midazolam. Despite their proven utility, these agents often fail to effectively terminate behavioral and electrographic seizure activity (Mayer et al., 2002). A recent large-scale clinical study found that about 25% and 36% of subjects with status epilepticus did not respond to intramuscular (IM) midazolam and intravenous (IV) lorazepam, respectively (Silbergleit et al., 2012). The primary mechanism of action of benzodiazepines is positive allosteric modulation of GABA_A receptors. However, as seizures progress, benzodiazepine-sensitive synaptic GABA_A receptors are actively internalized and become functionally inactive (Naylor et al., 2005; Goodkin et al., 2007; Jacob et al., 2008). This phenomenon is believed to underlie the refractoriness to benzodiazepines that occurs in prolonged status epilepticus. Benzodiazepine-insensitive GABA_A receptors containing $\alpha 4$ and δ subunits do not internalize with prolonged seizure

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Disclosure

M.A.R. is a consultant and C.M.L. and K.R. are employees of Sage Therapeutics. The remaining authors have no conflicts of interest. 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.

activity (Goodkin et al., 2008). GABA_A receptors containing 4 subunits can mediate phasic synaptic inhibition (Sun et al., 2007; Liang et al., 2008) whereas receptors containing 5 subunits (including receptors with both 4 and 5 subunits) are localized extrasynaptically and are believed to mediate tonic inhibition (Stell et al., 2003). Extrasynaptic GABA_A receptors containing 4 subunits therefore represent an attractive target for the treatment of benzodiazepine-resistant status epilepticus. Neuroactive steroid positive allosteric modulators (PAMs) of GABA_A receptors, such as allopregnanolone (an endogenous neuroactive steroid referred to as a “neurosteroid”), enhance both synaptic and extrasynaptic GABA_A receptors including those containing 4 (Belelli et al., 2002; Stell et al., 2003) and might be effective in the treatment of benzodiazepine-resistant status epilepticus.

Neurosteroid PAMs are cholesterol derivatives synthesized in the periphery and in situ in the nervous system that have been implicated as endogenous regulators of diverse behavioral functions (Do Rego et al., 2009). Neurosteroids also play a role in the control of seizure susceptibility in certain circumstances (Reddy and Rogawski, 2012). Although neurosteroid PAMs are derived from hormonally active steroids such as progesterone that act on nuclear hormone receptors, neurosteroids themselves do not have classical nuclear hormone receptor activity (Paul and Purdy, 1992). Peripherally produced neurosteroids can readily diffuse across the blood-brain barrier to engage central targets. Neurosteroid-based drugs have been studied as therapeutic agents, including as an orally administered drug (ganaxolone) for the chronic treatment of epilepsy (Bialer et al., 2013), although no such agent is currently approved for human use.

Allopregnanolone Confers Seizure Protection in Diverse Models

Allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one), a prototypic GABA_A receptor PAM neurosteroid, is a pregnane-ring steroid that is synthesized in endocrine tissues (ovary and adrenal) and brain (Corpéchet et al., 1993), by the sequential reduction of progesterone on its A-ring by 5 α -reductase and 3 α -hydroxysteroid oxidoreductase isoenzymes (Reddy and Rogawski, 2012). Basal circulating allopregnanolone levels are generally <1 nM in women in the follicular phase of the menstrual cycle and in men (Timby et al., 2006; Girdler et al., 2012) but increase to 1–4 nM in women during the luteal phase of the menstrual cycle (Genazzani et al., 2002; Nyberg et al., 2007) and to 20–200 nM during pregnancy (Luisi et al., 2000; Pařízek et al., 2005). Exogenously administered allopregnanolone (at doses as low as ~1 mg/kg) confers seizure protection in a range of models in which seizures are induced acutely by chemoconvulsants and electrical stimulation (Table 1). In addition, allopregnanolone is highly potent against fully kindled seizures in the amygdala kindling model of temporal lobe epilepsy. The seizure and epilepsy models in which allopregnanolone is effective are generally those in which other GABA_A receptor PAMs, such as benzodiazepines, confer seizure protection. At doses up to 300 mg/kg, allopregnanolone was not effective in the mouse maximal electroshock model (Belelli et al., 1989; Kokate et al., 1994), which is a model where GABA_A receptor PAMs are weak or ineffective. While tolerance to certain of the behavioral actions of GABA_A receptor PAM neuroactive steroids can occur (Turkmen et al., 2011), studies with the allopregnanolone analogs pregnanolone (3 α -hydroxy-5 α -pregnan-20-one) and ganaxolone (3 α -methyl-allopregnanolone) have indicated that tolerance does not occur to the anticonvulsant actions of neuroactive steroids (Kokate et al., 1998; Reddy and Rogawski, 2000). Tolerance was also not observed in long-term clinical trials with ganaxolone in the treatment of partial seizures (Bialer et al., 2013). Lack of anticonvulsant tolerance is a major pharmacological characteristic that differentiates GABA_A receptor PAM neuroactive steroids from benzodiazepines.

Anticonvulsant Activity of IV and IM Allopregnanolone

Although allopregnanolone is well recognized to be a highly effective antiseizure agent, the majority of studies have administered allopregnanolone via the subcutaneous (SC) or intraperitoneal (IP) routes (Belelli et al., 1989; Kokate et al., 1994, 1996; Kaminski et al., 2004). For human use in the acute management of status epilepticus, a treatment agent would be dosed IV or IM. Therefore, we recently studied IV and IM allopregnanolone using a parenteral formulation with the solubilizing agent sulfobutyl ethers β -cyclodextrin sodium salts (Captisol®, Ligand Pharmaceuticals, La Jolla, CA) in 0.9% saline (Zolkowska et al., 2013). The formulation of allopregnanolone we used is currently approved for investigational use by the U.S. Food and Drug Administration under IND 111,085. In the 6 Hz seizure model in mice, we found that 0.5 and 1.5 mg/kg allopregnanolone IV conferred seizure protection in 50% and 100% of animals at 1 min after dosing. The protective effect declined rapidly and was no longer evident at 15 and 30 min after the IV bolus with the low and higher doses, respectively. Similarly, allopregnanolone IV at doses as low as 0.25 mg/kg delayed the onset of pentylenetetrazol (PTZ)-induced seizures in mice. In rats we found that allopregnanolone IV at a dose of 0.5 mg/kg but not at 0.1 mg/kg caused a marked prolongation in the onset of PTZ-induced seizures. Allopregnanolone IV produces very rapid seizure protection in these models but the duration of action is short. Allopregnanolone IM was similarly effective in the 6 Hz model in mice: 1.5, 3 and 6 mg/kg doses protected 50%, 90% and 100% of animals, respectively. Seizure protection peaked at 5–10 min and persisted for up to 60 min. Allopregnanolone IM at doses as low as 0.5 mg/kg delayed the onset of PTZ-induced seizures in mice. Allopregnanolone is highly potent when administered IM and the onset and duration of action is somewhat longer than with IV administration.

Allopregnanolone in Adult Status Epilepticus Models

The muscarinic cholinergic agonist pilocarpine causes persistent motor seizures that are well recognized as a model of status epilepticus (Turski et al., 1987). Pretreatment with allopregnanolone (1–20 mg/kg, IP, 20 min before pilocarpine) prevented the development pilocarpine-induced status epilepticus and lethality in mice in a dose-dependent fashion, with ED₅₀ of 7.0 mg/kg (Kokate et al., 1996). In addition, 15 mg/kg pregnanolone was effective in terminating ongoing status epilepticus 15 min after pilocarpine. In 50% of mice the seizures were aborted completely whereas in the remainder seizure severity was reduced but not completely eliminated. A higher dose of pregnanolone (30 mg/kg) abolished ongoing status epilepticus in all animals but caused marked sedation in 63% of the animals. Allopregnanolone (3–50 mg/kg, IP) also caused a dose-dependent delay in the onset of kainic acid-induced limbic seizures (Kokate et al., 1996), another well-recognized chemoconvulsant model of status epilepticus (see Fritsch et al., 2010). Because kainic acid has a prolonged duration of action, it was necessary to administer a second dose of allopregnanolone 1 h after the first to reliably obtain protection of all animals from the occurrence of kainic acid-induced status epilepticus. A statistically-significant delay in the onset of kainic acid-induced seizures occurred with an allopregnanolone dose of 10 mg/kg administered according to this repeat-dose schedule and at higher doses all animals were protected.

Efficacy of Allopregnanolone in a Diazepam-Resistant Status Epilepticus Model

To assess the potential of allopregnanolone for the treatment of benzodiazepine resistant status epilepticus, we utilized a pharmacoresistant kainic acid pediatric model (Lossin et al., 2013). In P9 rats, status epilepticus was induced by treatment with kainic acid (2 mg/kg, IP).

Status epilepticus was defined as motor seizures of modified Racine score > 2, with interruptions of less than 10 s between seizures. Behavioral seizure activity was assessed at early (30–35 min) and late (70–75 min) time points after kainic acid treatment. Electrographic seizures were recorded with a 3-channel EEG/EMG monitoring system. When administered 10 min after kainic acid, animals treated with either diazepam (5 mg/kg, IP) or allopregnanolone (30 mg/kg, IP) were completely status epilepticus free at the early and late observation times (Fig. 1). In contrast, 65% and none of vehicle-treated animals were status epilepticus free at the two time points. However, when the treatments were administered 40 min after kainic acid, only 25% of the animals that had received diazepam were status epilepticus free at the late observation time whereas all of the animals that had received allopregnanolone were status epilepticus free at this time point. Assessment of behavioral seizures may be confounded by the sedative action of allopregnanolone at the relatively high dose used in this study. However, EEG recordings confirmed the ability of allopregnanolone to terminate electrographic seizure discharges. These preliminary studies indicate that allopregnanolone may be effective in the treatment of benzodiazepine-resistant status epilepticus.

Allopregnanolone and Neural Injury

Endogenous allopregnanolone has a role in the physiology of diverse behavioral functions and dysregulation of allopregnanolone has been implicated in various neurological and psychiatric conditions (Schüle et al., 2011). In particular, changes in allopregnanolone levels may play a role in the fluctuations in seizure susceptibility that occur in catamenial epilepsy, in response to stress, and in other clinical situations (Reddy and Rogawski, 2012). In addition, allopregnanone has neuroprotective properties (Borowicz et al., 2011), for example in models of traumatic brain injury (Djebaili et al., 2005) and stroke (Sayeed et al., 2006). Of particular relevance here is the ability of allopregnanolone to attenuate neuronal damage in the hippocampus following kainic acid treatment (Le kiewicz et al., 1997; Ciriza et al., 2004). While the neuroprotection observed in this situation could be due to antiseizure effects, it is noteworthy that in non-seizure related brain injury, benzodiazepines may not confer neuroprotective effects as is obtained with neuroactive steroids in such brain injury models (Kuhmonen et al., 2002; Davies et al., 2004). Therefore, allopregnanolone may have unique neuroprotective properties not shared by other GABA_A receptor PAMs. In sum, allopregnanolone may confer benefit in the treatment of status epilepticus by stopping seizures and also conceivably by protecting against seizure-induced neural injury.

Conclusions

Exogenously administered allopregnanolone has broad-spectrum anticonvulsant activity in diverse animal seizure models, including models of status epilepticus. Evidence presented here indicate that it could be useful in the treatment of benzodiazepine-resistant status epilepticus. Recently, IV allopregnanolone was administered under an emergency use IND to treat an individual with prolonged super-refractory status epilepticus (Vaitkevicius et al., 2013). This patient was successfully weaned from burst suppression drugs, the EEG profile normalized, and he is now seizure free. This case in combination with the available preclinical data suggest that parenteral allopregnanolone is worthy of investigation for the treatment of established refractory status epilepticus. Benzodiazepine resistance is likely to be a major cause of failure in the initial management of status epilepticus according to established treatment algorithms (Bleck, 2005). Therefore, allopregnanolone could also be useful as a first or second line agent.

Acknowledgments

This work was supported by grants from People Against Childhood Epilepsy (P.A.C.E.) and the Children's Miracle Network. Financial support was also provided by grants from the National Institute of Neurological Disorders and Stroke (NS072094, NS079202).

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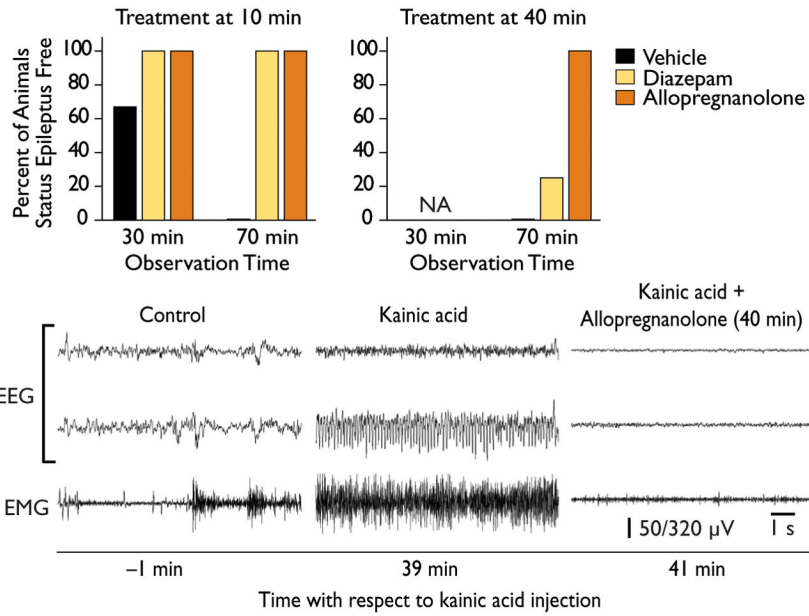


Figure 1. Comparison of diazepam and allopregnanolone in a pediatric rat status epilepticus model. Male P9 Sprague-Dawley rat pups were injected with kainic acid (2 mg/kg, IP), which reproducibly induced behavioral seizures, consisting of unilateral or bilateral limb or axial clonus, and generalized tonic-clonic convulsions. The animals received diazepam (5 mg/kg, IP) or allopregnanolone (30 mg/kg, IP) 10 or 40 min after kainic acid treatment. Animals were assessed for the presence or absence of seizures at 30 min (early) and 70 min (late) after the kainic acid injection. There were 5–93 animals in each of the early treatment groups and 5–17 animals in the late treatment groups. NA, not applicable. For acquisition of the EEG/EMG recordings, P6 rat pups were fitted with a head-mounted preamplifier connected through a low-torque commutator to a Data Conditioning and Acquisition System (Pinnacle Technology, Lawrence, KS) interfaced to a PC. Anterior and posterior right epidural EEG electrodes were used with ground and reference electrodes on left; EMG leads were implanted in neck muscles. Animals were treated with kainic acid (3 mg/kg) at time 0 min. Allopregnanolone (30 mg/kg, IP) was administered at 40 min. The EEG and EMG records shown were acquired at the indicated times. Allopregnanolone rapidly suppressed kainic acid-induced seizure activity. Top voltage scale value refers to EEG; bottom voltage scale value refers to EMG.

Table 1

Efficacy of Allopregnanolone in Rodent Seizure and Epilepsy Models

Model	Species	Activity	Literature Citation
<i>Acute Seizure Models</i>			
PTZ	Mouse (male)	ED ₅₀ =18.8 ± 1.1 mg/kg, IP ED ₅₀ =13.7 (10.1–18.7) mg/kg, IP ED ₅₀ =2.8 mg/kg, IP ED ₅₀ =2.27 (1.42–3.66) mg/kg, SC	Belelli et al. (1989); Kokate et al. (1994); Wieland et al. (1995); Gasior et al. (1997)
PTZ	Rat (female)	ED ₅₀ =2.14 (1.10–4.15) mg/kg, SC	Reddy and Rogawski (2001)
PTZ	Rat* (Male)	Minimum dose to reduce generalized tonic-clonic seizures: 10 mg/kg (suspension), IP	Mareš et al. (2006)
Bicuculline	Mouse (male)	ED ₅₀ =4.1 ± 1.7 mg/kg, IP	Belelli et al. (1989)
Picrotoxin	Mouse (male)	ED ₅₀ =31.7 ± 1.1 mg/kg, IP	Belelli et al. (1989)
Cocaine	Mouse (male)	ED ₅₀ =4.77 (2.27–10.0) mg/kg, SC	Gasior et al. (1997)
NMDA	Mouse (male)	Significant delay to seizure onset: 30 mg/kg, IP Significant increase in NMDA CD ₅₀ and LD ₅₀ : 5 mg/kg, IP	Kokate et al. (1996); Budziszewska et al. (1998)
6 Hz	Mouse (male)	ED ₅₀ =14.2 (10.4–19.4) mg/kg, IP	Kaminski et al. (2004)
<i>Epilepsy Models</i>			
Amygdala kindled seizures	Rat (female)	ED ₅₀ =1.1 mg/kg, IP	Lonsdale et al. (2007)
Amygdala kindled seizures	Mouse (female)	Inhibits behavioral seizure stage and afterdischarge duration: 1–10 mg/kg, IP	Reddy et al. (2012)
<i>Status Epilepticus Models</i>			
Pilocarpine	Mouse (male)	ED ₅₀ =7.0 (3.9–11.4) mg/kg, IP	Kokate et al. (1996)
Kainate	Mouse (male)	Significant delay to seizure onset: 10 mg/kg, IP	Kokate et al. (1996)
Kainate	Mouse	Significant increase in kainate CD ₅₀ and LD ₅₀ : 10 mg/kg, IP	Le kiewicz et al. (1997)
Kainate	Rat	Reduced seizure incidence: 4–8 mg/kg, SC	Frye and Bayon (1999)
Kainate	Rat (female) [†]	Significant delay to seizure onset: 4 mg/kg, SC	Frye and Scalise (2000)
Perforant path stimulation	Rat (male)	Reduced incidence and duration of partial seizures: 2.5 mg/kg, SC	Frye (1995)
Perforant path stimulation	Rat (female) [†]	Reduced incidence and duration of partial seizures: 4 mg/kg, SC	Frye and Scalise (2000)

Allopregnanolone is inactive in the maximal electroshock test and strychnine model at doses <300 mg/kg, IP (Belelli et al., 1989; Kokate et al., 1994). Protection against NMDA-induced seizures required two doses; a single dose was inactive (Kokate et al., 1996; Gasior et al., 1997). Range given in parentheses after ED₅₀ values represent 95% confidence limits. ED₅₀, dose-producing seizure protection in 50% of animals; range given in parentheses after ED₅₀ values represent 95% confidence limits. CD₅₀, dose-producing seizures in 50% of animals. LD₅₀, dose causing lethality in 50% of animals.

* 7–90 days of age.

[†] Ovariectomized.