Epilepsia, 54(Suppl. 6):93–98, 2013
doi: 10.1111/epi.12289

Neuroactive steroids for the treatment of status epilepticus

***Michael A. Rogawski, †Carlos M. Loya, †Kiran Reddy, *Dorota Zolkowska, and *Christoph Lossin**

***Department of Neurology, School of Medicine, University of California, Davis, Sacramento, California, U.S.A.; and †Sage Therapeutics, Cambridge, Massachusetts, U.S.A.**

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.

KEY WORDS: Refractory status epilepticus, Seizure, Allopregnanolone, Neurosteroid, Allosteric modulator, Extrasynaptic $GABA_A$ receptor.

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 in status epilepticus (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 α4 and δ subunits do not internalize with prolonged seizure activity (Goodkin et al., 2008). $GABA_A$ receptors containing α4 subunits can mediate phasic synaptic inhibition (Sun et al., 2007; Liang et al., 2008), whereas receptors containing δ subunits (including receptors with both α4 and δ subunits) are localized extrasynaptically and are believed to mediate tonic inhibition (Stell et al., 2003). Extrasynaptic $GABA_A$ receptors containing α4δ 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 α4δ

Address correspondence to Michael A. Rogawski, Department of Neurology, School of Medicine, University of California, Davis, 4860 Y Street, Suite 3700, Sacramento, CA 95817, U.S.A. E-mail: rogawski@ucdavis.edu

(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 & 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 & 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 (3α-hydroxy-5α-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 (Corpéchot et al., 1993), by the sequential reduction of progesterone on its A-ring by 5α-reductase and 3α-hydroxysteroid oxidoreductase isoenzymes (Reddy & 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 (Lonsdale & Burnhan, 2007; Reddy et al., 2012). 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. Although 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 (3α-hydroxy-5β-pregnan-20-one) and ganaxolone (3β-methyl-allopregnanolone) have indicated that tolerance does not occur to the anticonvulsant actions of neuroactive steroids (Kokate et al., 1998; Reddy & 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 pharmacologic 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, U.S.A.) 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 (FDA) 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, respectively, 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 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.

Table 1. Efficacy of allopregnanolone in rodent seizure and epilepsy models

Model	Species	Activity	Literature citation
Acute seizure models			
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[a] (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)
Epilepsy models			
Amygdala kindled seizures	Rat (female)	ED_{50} = 1.1 mg/kg, IP	Lonsdale and Burnham (2007)
Amygdala kindled seizures	Mouse (female)	Inhibits behavioral seizure stage and afterdischarge duration: 1–10 mg/kg, IP	Reddy et al. (2012)
Status epilepticus models			
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	Leśkiewicz et al. (1997)
Kainate	Rat	Reduced seizure incidence: 4–8 mg/kg, SC	Frye and Bayon (1999)
Kainate	Rat (female)[b]	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)[b]	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). ED_{50}, dose-producing seizure protection in 50% of animals; range given in parentheses after ED_{50} values represent 95% confidence limits. CD_{50}, dose-producing seizures in 50% of animals. LD_{50}, dose causing lethality in 50% of animals.

[a]7–90 days of age.

[b]Ovariectomized.

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 ED_{50} of 7.0 mg/kg (Kokate et al., 1996). In addition, 15 mg/kg pregnanolone (which is slightly less potent than allopregnanolone in the PTZ model) (Kokate et al., 1994) 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 <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 electroencephalography/electromyography (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

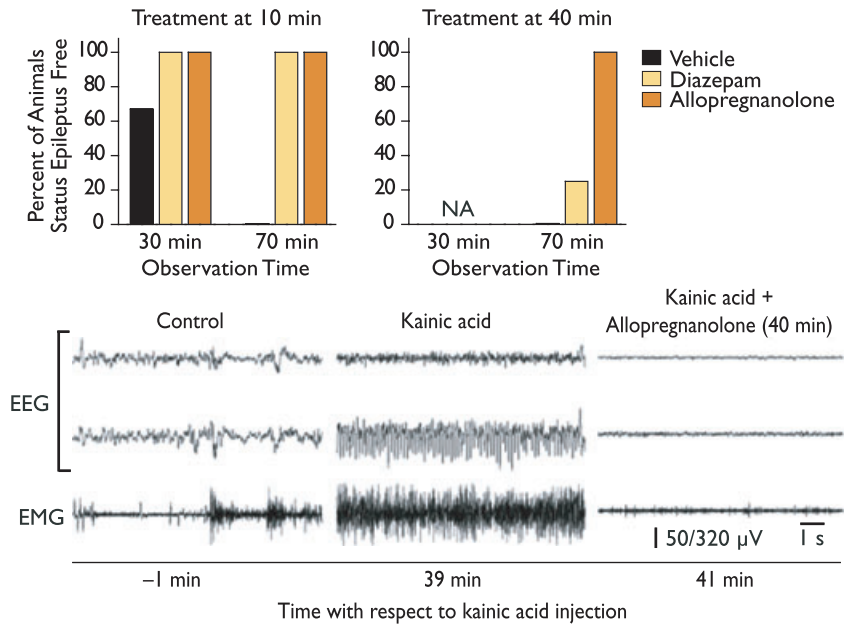

Figure 1.
Comparison of diazepam and allopregnanolone in a pediatric rat status epilepticus model. Male P9 Sprague–Dawley rat pups were injected with kainic acid (2 mg/kg, IP), which reproducibly induced behavioral seizures, consisting of unilateral or bilateral limb or axial clonus, and generalized 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, U.S.A.) interfaced to a personal computer. 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.

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 neurologic 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 & Rogawski, 2012). In addition, allopregnanolone 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 (Leśkiewicz et al., 1997; Ciriza et al., 2004). Although the neuroprotection observed in this situation could be caused by 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 indicates 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 superrefractory 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 suggests 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).

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.

References

Belelli D, Bolger MB, Gee KW. (1989) Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. *Eur J Pharmacol* 166:325–329.

Belelli D, Casula A, Ling A, Lambert JJ. (2002) The influence of subunit composition on the interaction of neurosteroids with $GABA_A$ receptors. *Neuropharmacology* 43:651–661.

Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. (2013) Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI). *Epilepsy Res* 103:2–30.

Bleck TP. (2005) Refractory status epilepticus. *Curr Opin Crit Care* 11:117–120.

Borowicz KK, Piskorska B, Banach M, Czuczwar SJ. (2011) Neuroprotective actions of neurosteroids. *Front Endocrinol (Lausanne)* 2:50.

Budziszewska B, Siwanowicz J, Leśkiewicz M, Jaworska-Feil L, Lasoń W. (1998) Protective effects of neurosteroids against NMDA-induced seizures and lethality in mice. *Eur Neuropsychopharmacol* 8:7–12.

Ciriza I, Azcoitia I, Garcia-Segura LM. (2004) Reduced progesterone metabolites protect rat hippocampal neurones from kainic acid excitotoxicity in vivo. *J Neuroendocrinol* 16:58–63.

Corpéchot C, Young J, Calvel M, Wehrey C, Veltz JN, Touyer G, Mouren M, Prasad VVK, Banner C, Sjövall J, Baulieu EE, Robel P. (1993) Neurosteroids: 3α-hydroxy-5α-pregnan-20-one and its precursors in the brain, plasma, and steroidogenic glands of male and female rats. *Endocrinology* 133:1003–1009.

Davies LM, MacLellan CL, Corbett DR, Colbourne F. (2004) Post-ischemic diazepam does not reduce hippocampal CA1 injury and does not improve hypothermic neuroprotection after forebrain ischemia in gerbils. *Brain Res* 1013:223–229.

Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. (2005) The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma* 22:106–118.

Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H. (2009) Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front Neuroendocrinol* 30:259–301.

Fritsch B, Stott JJ, Joelle Donofrio J, Rogawski MA. (2010) Treatment of early and late kainic acid-induced status epilepticus with the noncompetitive AMPA receptor antagonist GYKI 52466. *Epilepsia* 51:108–117.

Frye CA. (1995) The neurosteroid 3α,5α-THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res* 696:113–120.

Frye CA, Bayon LE. (1999) Prenatal stress reduces the effectiveness of the neurosteroid 3α,5α-THP to block kainic-acid-induced seizures. *Dev Psychobiol* 34:227–234.

Frye CA, Scalise TJ. (2000) Anti-seizure effects of progesterone and 3α,5α-THP in kainic acid and perforant pathway models of epilepsy. *Psychoneuroendocrinology* 25:407–420.

Gasior M, Carter RB, Goldberg SR, Witkin JM. (1997) Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjunction with diazepam. *J Pharmacol Exp Ther* 282:543–553.

Genazzani AD, Luisi M, Malavasi B, Strucchi C, Luisi S, Casarosa E, Bernardi F, Genazzani AR, Petraglia F. (2002) Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroid, during the menstrual cycle and in amenorrheic subjects. *Eur J Endocrinol* 146:347–356.

Girdler SS, Lindgren M, Porcu P, Rubinow DR, Johnson JL, Morrow AL. (2012) A history of depression in women is associated with an altered GABAergic neuroactive steroid profile. *Psychoneuroendocrinology* 37:543–553.

Goodkin HP, Sun C, Yeh JL, Mangan PS, Kapur J. (2007) GABAA receptor internalization during seizures. *Epilepsia* 48(Suppl. 5):109–113.

Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. (2008) Subunit-specific trafficking of $GABA_A$ receptors during status epilepticus. *J Neurosci* 28:2527–2538.

Jacob TC, Moss SJ, Jurd R. (2008) $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci* 9:331–343.

Kaminski RM, Livingood MR, Rogawski MA. (2004) Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia* 45:864–867.

Kokate TG, Svensson BE, Rogawski MA. (1994) Anticonvulsant activity of neurosteroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. *J Pharmacol Exp Ther* 270:1223–1229.

Kokate TG, Cohen AL, Karp E, Rogawski MA. (1996) Neuroactive steroids protect against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. *Neuropharmacology* 35:1049–1056.

Kokate TG, Yamaguchi S, Pannell LK, Rajamani U, Carroll DM, Grossman AB, Rogawski MA. (1998) Lack of anticonvulsant tolerance to the neuroactive steroid pregnanolone in mice. *J Pharmacol Exp Ther* 287:553–558.

Kuhmonen J, Lukkarinen J, Gröhn O, Jolkkonen J, Sivenius J. (2002) Diazepam does not reduce infarct size in rats subjected to transient occlusion of the middle cerebral artery when normothermia is maintained. *J Pharm Pharmacol* 54:1565–1569.

Leśkiewicz M, Budziszewska B, Jaworska-Feil L, Lasoń W. (1997) Effects of neurosteroids on kainate-induced seizures, neurotoxicity and lethality in mice. *Pol J Pharmacol* 49:411–417.

Liang J, Suryanarayanan A, Chandra D, Homanics GE, Olsen RW, Spigelman I. (2008) Functional consequences of $GABA_A$ receptor α4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. *Alcohol Clin Exp Res* 32:19–26.

Lonsdale D, Burnham WM. (2007) The anticonvulsant effects of allopregnanolone against amygdala-kindled seizures in female rats. *Neurosci Lett* 411:147–151.

Lossin C, Shahangian S, Rogwski MA. (2013) Allopregnanolone treatment in a rat pediatric status epilepticus model: comparison with diazepam. *Epilepsy Curr* 13(Suppl. 1):418 (Abst 3.220).

Luisi S, Petraglia F, Benedetto C, Nappi RE, Bernardi F, Fadalti M, Reis FM, Luisi M, Genazzani AR. (2000) Serum allopregnanolone levels in pregnant women: changes during pregnancy, at delivery, and in hypertensive patients. *J Clin Endocrinol Metab* 85:2429–2433.

Mareš P, Mikulecká A, Haugvicová R, Kasal A. (2006) Anticonvulsant action of allopregnanolone in immature rats. *Epilepsy Res* 70:110–117.

Mayer SA, Claassen J, Lokin J, Mendelsohn F, Dennis LJ, Fitzsimmons BF. (2002) Refractory status epilepticus: frequency, risk factors, and impact on outcome. *Arch Neurol* 59:205–210.

Naylor DE, Liu H, Wasterlain CG. (2005) Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci* 25:7724–7733.

Nyberg S, Bäckström T, Zingmark E, Purdy RH, Poromaa IS. (2007) Allopregnanolone decrease with symptom improvement during placebo and gonadotropin-releasing hormone agonist treatment in women with severe premenstrual syndrome. *Gynecol Endocrinol* 23:257–266.

Pařízek A, Hill M, Kancheva R, Havlíková H, Kancheva L, Cindr J, Pašková A, Pouzar V, Černý I, Drbohlav P, Hájek Z, Stárka L. (2005) Neuroactive pregnanolone isomers during pregnancy. *J Clin Endocrinol Metab* 90:395–403.

Paul SM, Purdy RH. (1992) Neuroactive steroids. *FASEB J* 6:2311–2322.

Reddy DS, Rogawski MA. (2000) Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther* 295:1241–1248.

Reddy DS, Rogawski MA. (2001) Enhanced anticonvulsant activity of neuroactive steroids in a rat model of catamenial epilepsy. *Epilepsia* 42:337–344.

Reddy DS, Rogawski MA (2012) Neurosteroids — Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy In Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (Eds) *Jasper's Basic Mechanisms of the Epilepsies*. Contemporary Neurology Series 80, Oxford University Press, New York, pp. 984–1002.

Reddy DS, Gould J, Gangisetty O. (2012) A mouse kindling model of perimenstrual catamenial epilepsy. *J Pharmacol Exp Ther* 341:784–789.

Sayeed I, Guo Q, Hoffman SW, Stein DG. (2006) Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med* 47:381–389.

Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. (2011) Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience* 191:55–77.

Silbergleit R, Durkalski V, Lowenstein D, Conwit R, Pancioli A, Palesch Y, Barsan W, Investigators NETT. (2012) Intramuscular versus intravenous therapy for prehospital status epilepticus. *N Engl J Med* 366:591–600.

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors. *Proc Natl Acad Sci USA* 100:14439–14444.

Sun C, Mtchedlishvili Z, Erisir A, Kapur J. (2007) Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the α4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J Neurosci* 27:12641–12650.

Timby E, Balgård M, Nyberg S, Spigset O, Andersson A, Porankiewicz-Asplund J, Purdy RH, Zhu D, Bäckström T, Poromaa IS. (2006) Pharmacokinetic and behavioral effects of allopregnanolone in healthy women. *Psychopharmacology* 186:414–424.

Turkmen S, Backstrom T, Wahlstrom G, Andreen L, Johansson IM. (2011) Tolerance to allopregnanolone with focus on the GABA-A receptor. *Br J Pharmacol* 162:311–327.

Turski L, Cavalheiro EA, Czuczwar SJ, Turski WA, Kleinrok Z. (1987) The seizures induced by pilocarpine: behavioral, electroencephalographic and neuropathological studies in rodents. *Pol J Pharmacol Pharm* 39:545–555.

Vaitkevicius H, Ng M, Moura L, Rosenthal E, Westover MB, Rosand J, Rogawski MA, Reddy K, Cole AJ (2013) Successful allopregnanolone treatment of new onset refractory status epilepticus (NORSE) syndrome: first in man experience. The 4th London-Innsbruck Colloquium on Status Epilepticus and Acute Seizures. Final Programme. p. 124 (Abstract P29). http://www.statusepilepticus2013.eu/

Wieland S, Belluzzi JD, Stein L, Lan NC. (1995) Comparative behavioral characterization of the neuroactive steroids 3α-OH,5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents. *Psychopharmacology* 118:65–71.

Zolkowska D, Dhir A, Cooke GR, Wu C, Zhu L, Wulff H, Rogawski MA. (2013) Anticonvulsant activity of intravenous and intramuscular allopregnenalone. *Epilepsy Curr* 13(Suppl. 1):11 (Abst 1.023).