Ganaxolone: Mechanism of Action and Pharmacology

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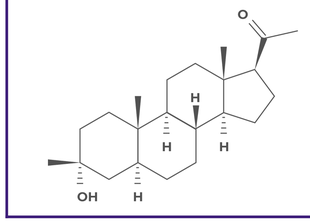
Introduction

- Neuroactive steroids (NAS), both synthetic and endogenous, are cholesterol-derived compounds that rapidly alter neuronal excitability by binding to membrane-bound receptors
- Anticonvulsant and antiepileptogenic properties of NAS are related to their pharmacological actions as positive allosteric modulators of γ-aminobutyric acid type A ($GABA_A$) receptors and are not associated with activating classical steroid hormone receptors that regulate gene transcription
- Ganaxolone (GNX) is a synthetic NAS in clinical development for both acute and chronic seizure disorders
- GNX is the 3β-methylated synthetic analog of the naturally occurring neurosteroid, allopregnanolone (**Figure 1**)[1]
  - 3β-methyl substitution renders GNX orally bioavailable
  - GNX does not convert back to a metabolite with activity at the nuclear progesterone receptor

Figure 1. Chemical Structure of Ganaxolone



Mechanism of Action

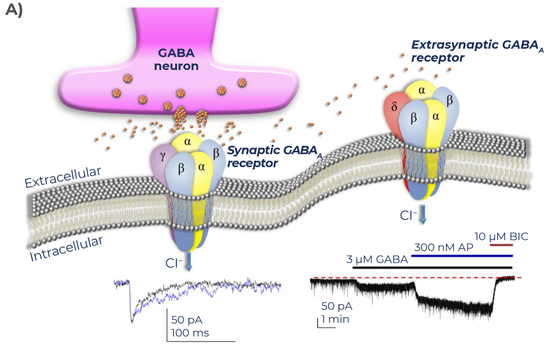
- GNX is a positive allosteric modulator of $GABA_A$ receptors[1] (**Figure 2**)
  - GNX enhances the activity of the neurotransmitter GABA through a binding site distinct from that of benzodiazepines
- GNX modulates synaptic $GABA_A$ receptors (comprising α and γ subunits) and, unlike benzodiazepines, also modulates extrasynaptic $GABA_A$ receptors (comprising α and δ subunits) (**Figure 3A**)[2]
  - Tonic inhibition produced by activating the α- and δ-containing $GABA_A$ receptors leads to persistent conductance and dampening of network excitability[3]
  - GNX has similar receptor efficacy to allopregnanolone at both synaptic and extrasynaptic receptors (**Figure 3B**)[1,4]
- The $GABA_A$ receptor has been linked to several neurological conditions, including seizure disorders
  - The extrasynaptic receptor has emerged as an important drug target, which most available GABAergic drugs do not activate[5]

Figure 2. GABA-Evoked Currents in Xenopus Oocytes Expressing the Human $GABA_A$ $\alpha_1\beta_1\gamma_2L$ Receptor

Traces represent the response of GABA (30 μM) ± ganaxolone. Asterisks note a change in vertical calibration bar. GABA, γ-aminobutyric acid; $GABA_A$, γ-aminobutyric acid type A.
Reprinted from *J Pharmacol Exp Ther*, Carter RB, et al, 1997, 280(3), pp. 1284-1295.

Figure 3. Schematic of GABAergic Inhibition at Both Synaptic and Extrasynaptic Receptor Sites and Effect (EC_{50}) of NAS at Both Receptor Types

A)



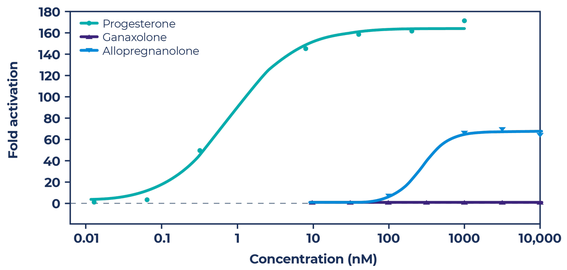
B)

	Synaptic receptor (α and γ subunits) (EC_{50}, nM)	Extrasynaptic receptor (α and δ subunits) (EC_{50}, nM)
GNX	213[a]	103
Allopregnanolone	149	75

(A) Schematic of GABAergic inhibition at both synaptic (phasic) and extrasynaptic (tonic) receptor sites. GNX enhances GABA-induced inhibitory signaling at both receptors. (B) EC_{50} values of GNX and allopregnanolone at both synaptic and extrasynaptic $GABA_A$ receptors. AP, allopregnanolone; BIC, bicuculline; GABA, γ-aminobutyric acid; $GABA_A$, γ-aminobutyric acid type A; GNX, ganaxolone.
[a]EC_{50} values range from 94 to 213 nM, depending on subunit composition.
Figure 3A republished with permission of *Psychopharmacology*, from "Neurosteroid interactions with synaptic and extrasynaptic GABA(A) receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability." Carver CM, Reddy DS; 230(2);2013. Permission conveyed through Copyright Clearance Center, Inc.

- GNX is inactive at a variety of alternative receptor classes, minimizing the potential for off-target effects[1]
  - GNX is not active at the nuclear progesterone receptor, unlike other neuroactive steroids (**Figure 4**)

Figure 4. Activity of Progesterone, Allopregnanolone, and Ganaxolone at the Nuclear Progesterone Receptor



Preclinical Efficacy

- GNX has been studied in multiple seizure-related animal models with a median effective dose that produces seizure protection in 50% of mice (ED_{50}) of ≈8 mg/kg (**Table 1**)

Table 1. Summary of Preclinical Efficacy of Ganaxolone in Various Seizure-Related Animal Models

Seizure model	ED_{50} value (mg/kg)	Source
Pentylenetetrazol	3.5	Reddy et al, 2000[6]
Pentylenetetrazol kindling	4.1	Gasior et al, 2000[7]
Bicuculline	4.6	Carter et al, 1997[1]
Flurothyl	5.0	Liptáková et al, 2000[8]
6 Hz	6.3	Kaminski et al, 2004[9]
Amygdala kindling	6.6	Reddy et al, 2010[10]
t-Butylbicycloorthobenzoate	11.7	Carter et al, 1997[1]
Aminophylline	11.5	Carter et al, 1997[1]
Cocaine kindling	17.0	Kaminski et al, 2003[11]
Maximal electroshock	29.7	Carter et al, 1997[1]
Alcohol withdrawal	<10	Nipper et al, 2019[12]
N-methyl-D-aspartate	>30	Carter et al, 1997[1]
Strychnine	>40	Carter et al, 1997[1]
Lithium-pilocarpine (status epilepticus)	6-9	Saporito et al, 2019[13]

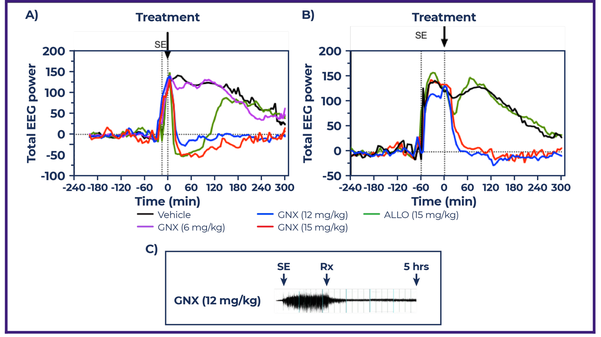
The ED_{50} value in the rotarod test of motor toxicity in mice was 33.4 mg/kg.
ED_{50}, median effective dose estimated to produce seizure protection in 50% of mice.

- GNX can be formulated for both acute, intravenous administration and chronic, oral administration

Acute administration for status epilepticus

- GNX was studied in a benzodiazepine-resistant lithium-pilocarpine-induced model of status epilepticus (SE) in rats
  - Clinically, benzodiazepines lose efficacy following prolonged seizures. A proposed mechanism is the internalization of synaptic $GABA_A$ receptors
- GNX rapidly and durably stopped seizures associated with SE (**Figure 5**)[13]
  - GNX blocked electrographic SE for up to 5 hours after SE onset
  - GNX was more potent and produced a more durable response than did allopregnanolone

Figure 5. Change in Total EEG Power in Animals Induced With Status Epilepticus and Treated With Vehicle



Total EEG power in animals induced with SE and treated with vehicle, ganaxolone, or allopregnanolone at (A) 15 minutes or (B) 60 minutes post-SE onset. (C) Representative EEG trace showing onset of SE and durable EEG suppression for up to 5 hours when dosed with GNX (12 mg/kg). ALLO, allopregnanolone; EEG, electroencephalogram; GNX, ganaxolone; Rx, treatment; SE, status epilepticus.

Chronic administration for epilepsies

- GNX does not exhibit tolerance following chronic administration in a preclinical study, allowing for the potential long-term durability of effect[14]
  - Chronic treatment with GNX did not significantly affect its antiseizure potency (acute ED_{50} = 3.8 mg/kg; chronic ED_{50} = 2.6 mg/kg)
  - Chronic treatment with diazepam shifted its ED_{50} ≈2-fold less potent (acute ED_{50} = 1.9 mg/kg; chronic ED_{50} = 3.7 mg/kg)

Conclusions

- **GNX is a positive allosteric modulator of $GABA_A$ receptors**
- **GNX exhibits effects similar to those of allopregnanolone at both synaptic and extrasynaptic receptors**
- **GNX does not affect other receptors, ion channels, or signaling systems, including activation of progesterone receptors**
- **$GABA_A$ receptors have been linked to several neurological disorders including epilepsies**
- **In preclinical studies, GNX is effective in blocking seizures evoked by a variety of epileptogenic agents**
- **GNX has been shown to be effective when administered after seizure onset**
- **Chronic administration of GNX in experimental models (or an experimental model) of epilepsy does not produce tolerance**

References

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