Blocking of GABA-AT Activity by OV329 Selectively Alters Tonic and Phasic Inhibition in Dentate Gyrus Granule Cells

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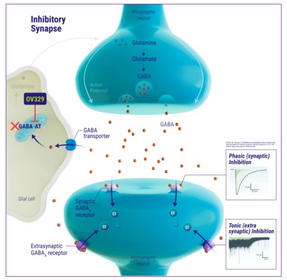
Background and Rationale

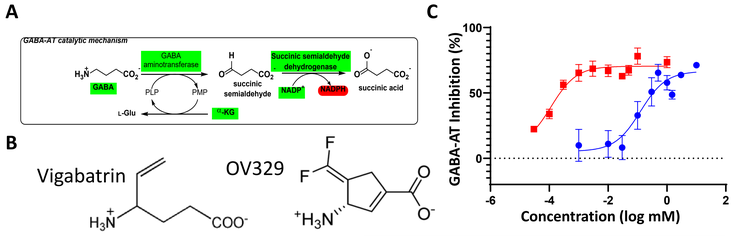
γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system (CNS) and is primarily catabolized by GABA amino transferase (GABA-AT). In patients with epilepsy, reduced CNS GABA levels are associated with poor seizure control. Increasing GABA levels through GABA-AT inhibition may improve seizure control. Currently, vigabatrin (VGB) is the only FDA-approved drug that blocks GABA-AT activity as a primary mechanism of action with proven efficacy in seizure reduction in up to 40% of chronically treated patients. However, clinical use of VGB in epilepsy is associated with retinal damage in chronically treated patients, limiting its use [1, 2].



OV329 is a novel, potent inactivator of GABA-AT [3] under development for the treatment of drug-resistant epilepsy. Here, we assessed how GABA-AT inhibition by OV329 modulates phasic and tonic GABAergic transmission in hippocampal dentate gyrus, a brain region intimately associated with temporal lobe epilepsy [4,5].

OV329 is a mechanism-based inhibitor of GABA-AT and is markedly more potent than vigabatrin. We hypothesize that OV329 will be efficacious at lower concentrations than vigabatrin in drug-resistant epilepsies, offering a broader therapeutic window and use potential.

Figure 1: OV329 is a Highly Potent Inactivator of GABA-AT



OV329 is the most potent inactivator of GABA-AT known to date with at least ~200x lower IC50 to GABA-AT than vigabatrin

	OV329	Vigabatrin	Fold Diff.
IC_{50}	0.11 μM	126 μM	~ 1000

A. Diagram showing the catabolic pathway of GABA via GABA-AT to succinic semialdehyde and succinic acid (PLP = pyridoxal-5′-phosphate). **B.** Structures of vigabatrin and OV329. **C.** GABA-AT enzyme activity was measured in the presence of OV329 and VGB using whole mouse brain lysates (8-12 wks). 20 μg of total protein was used for the assay with varying concentrations of OV329 & VGB. Note that the GABA-AT activity is inhibited in a dose-dependent manner for both OV329 and VGB. Compared to VGB, OV329 seems to be far more potent in inhibiting GABA-AT *in vitro*. All data points were minimally duplicated. n=3, mean ± SEM

References **1.** Wild JM, et al. CNS Drugs. 2009; 23(11):965-82. **2.** Maguire MJ, et al., Epilepsia, 2010. 51(12): 2423-31. **3.** Juncosa JI, et al. J Am Chem Soc. 2018;140(6): 2151-64. **4.** Coulter DA & Carlson GC (2007) Prog Brain Research 163:235–243. **5.** Farrant M & Nusser Z (2005) Nat Rev Neuroscience 6(3):215-229.

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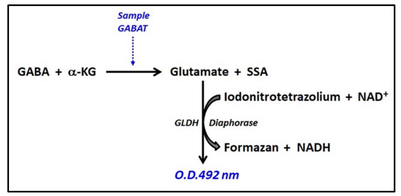
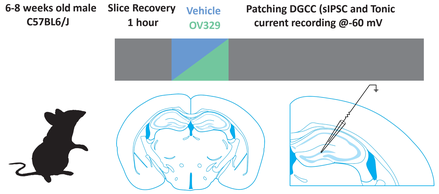
Methods

Figure 2: Principle of GABA-AT Activity Assay



The GABA-AT assay is based on sequential GABAT-mediated transamination reaction and glutamate dehydrogenase (GLDH) reaction, which couples the reduction of Iodonitrotetrazolium (INT) to formazan (EC = 18 $mM^{-1}cm^{-1}$ @ 492 nm), allowing for sensitive detection of GABAT enzyme activity in cell or tissue extracts. GABA-AT converts GABA to glutamate and the assay measures the conversion of glutamate to formazan through a colorimetric reaction. Glutamate being abundant in the brain may add noise to the assay. Therefore, it is critical to measure the ΔOD between the control (no substrate) and reaction (with substrate) sample.

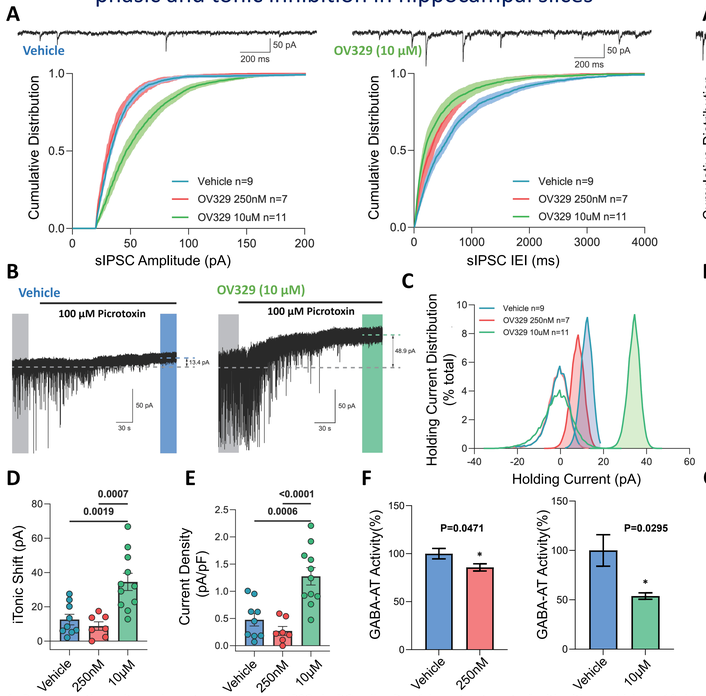
Figure 3: Electrophysiology Experiment



The effect of OV329 on both forms of GABAergic inhibition was measured using whole-cell patch clamp electrophysiology in the Dentate Gyrus Granule Cell (DGGC) layer of 6- to 8-week-old male C57Bl6/J mice using acute slices and a repeated *in vivo dosing* paradigm. The acute effects of OV329 were tested on hippocampal brain slices after a 1-hour incubation with either PBS (vehicle), 0.25 or 10μM OV329. The *in vivo* multiple dosing (IP) paradigm was tested following 6 days of treatment with either vehicle or 5 mg/kg OV329. Electrophysiological recordings were carried out in DGCCs, and spontaneous inhibitory postsynaptic currents (sIPSC) were captured prior to the application of picrotoxin to block all $GABA_A$Rs and, measure the resulting shift in the holding current caused by the block of extrasynaptic $GABA_A$ receptors. 5–7-week-old male C57Bl6/J mice were weighed and labeled with tail markings for identification purposes and received up to 6 days of repeated IP injections with volumes of 0.01 ml/g of Vehicle (pH 7.4 PBS) or 5 mg/kg OV329, or a single injection of 5 mg/kg OV329 at the same volume. Recordings were done at -60 mV in a voltage clamp configuration in the presence of the $GABA_B$R antagonist CGP54626 (1 μM). Appropriate statistical analysis e.g. One-way ANOVA was performed using GraphPad Prism and mentioned in the results section.

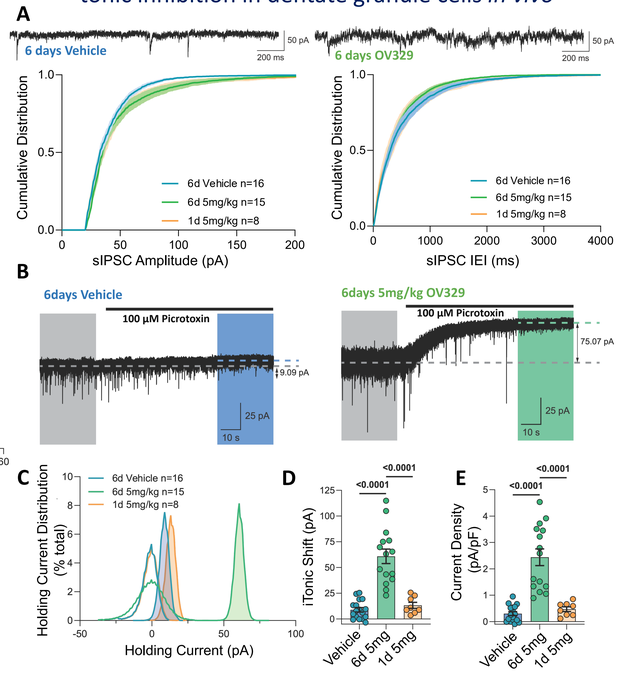
Results

Figure 4: Acute treatment of OV329 significantly increased phasic and tonic inhibition in hippocampal slices



A. Representative voltage clamp traces showing sIPSCs in DG cells after 1-hour treatment of with either vehicle or 10μM OV329. Cumulative distribution plot of sIPSCs amplitude and IEI showing significant increase in amplitude and frequency of sIPSCs after 10μM OV329 treatment compared to either vehicle or 250nM of OV329. **B.** Representative voltage-clamp traces showing a shift in holding current after 10μM OV329 following PTX application. The shift in the holding current caused by blockade of $GABA_A$Rs by PTX was due to extra-synaptic $GABA_A$R-mediated tonic current. **C.** All-points histograms show the baseline holding current and the holding current following PTX application. Compared to vehicle or low (250nM), high (10μM) OV329 significantly (one-way ANOVA) enhances tonic current shift **(D)** and mean tonic current density **(E)** in DGCC. **F.** GABA-AT activity measured from acute hippocampal slices after 1-hour treatment of OV329 shows significant (unpaired t- test) reduction; ~15% with 250nM and ~50% with 10μM OV329 treatment, n=8 (250nM), n=4 (10μM) slices from N=2 mice. Recordings were from Vehicle: n=9, 250 nM OV329: n=7, 10μM OV329: n=11 from N=3-5 mice

Figure 5: Repeat, low doses of OV329 significantly increased tonic inhibition in dentate granule cells *in vivo*



A. Representative voltage clamp traces showing sIPSC in DG cells after 6-days of treatment with either vehicle or 5 mg/kg OV329. Cumulative distribution plot of sIPSC amplitude and IEI showing compared to vehicle, no significant changes observed either after 1 or 6 days of OV329 treatment. **B.** Representative voltage-clamp traces showing a shift in holding current after 6days of OV329 following PTX application. The shift in the holding current caused by blockade of $GABA_A$Rs by PTX was due to extra-synaptic $GABA_A$R-mediated tonic current. **C.** All-points histograms show the baseline holding current and the holding current following PTX application. Compared to vehicle and single dose of OV329, multiple, daily dosing (5mg/kg X 6days) of OV329 significantly (one-way ANOVA) enhances tonic current shift **(D)** and mean tonic current density **(E)** in DGCC. Electrophysiological recordings were from Vehicle: n=16, 6-day OV329: n=15, 1day-OV329: n=8 from N=4-5 mice.

Conclusions

- **Acute treatment of high (10 μM) concentration of OV329 is sufficient to induce a significant change in phasic and tonic current within an hour, while low (250nM) concentration of OV329 is unable to elicit a similar change.**
- **Acute OV329 significantly inhibits GABA-AT activity in hippocampal slices in a dose-dependent manner and the extent of GABA-AT inhibition may underlie differential electrophysiological effect.**
- **Repeat dosing of OV329 (5 mg/kg/day for 6 days) significantly increases tonic GABA current without affecting phasic current while the effect on either phasic or tonic current was not observed after a single dose of 5mg/kg OV329.**
- **Electrophysiological data support the cumulative effect of OV329 on tonic inhibition is greatly enhanced due to repeat dosing of OV329.**

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