Novel first-in-class voltage-gated calcium channel positive allosteric modulators that stabilize the open state of the channel and have therapeutic potential to treat a variety of neuromuscular conditions

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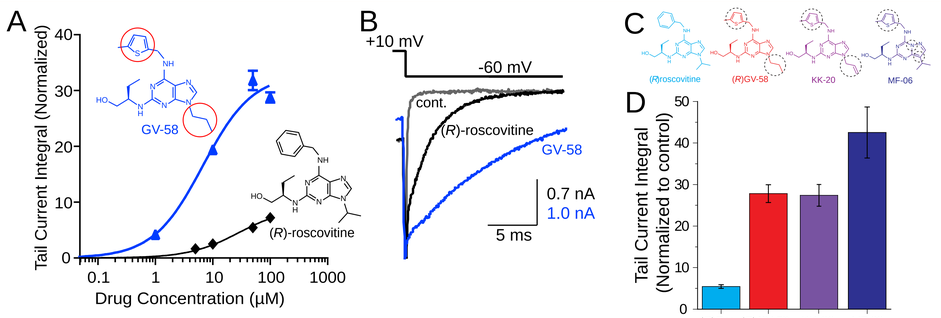
Introduction

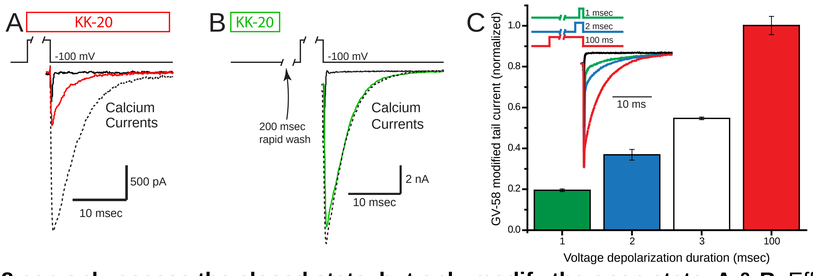
We have recently developed a series of novel, first-in-class, voltage-gated calcium channel (VGCC) positive allosteric gating modifiers that are selective for the types of VGCCs that regulate transmitter release at neuromuscular synapses (Cav2). These small molecules do not cross the blood-brain barrier well and have been shown to have therapeutic potential to treat a variety of neuromuscular diseases characterized by weakness of presynaptic origin (Lambert-Eaton myasthenic syndrome (LEMS) and Spinal Muscular Atrophy (SMA).

Development of novel Cav2 gating modifiers

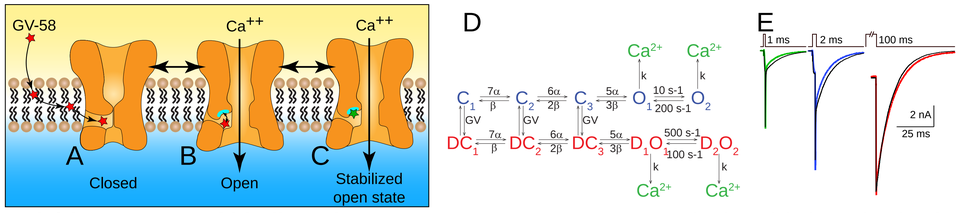


Comparison of the parent molecule (R)-roscovitine (Ros) with GV-58. A. GV-58 (changes from Ros circled) is more potent than Ros as a VGCC agonist (EC50 at Cav2.1 channels = 8.8 μM vs. 120 μM, respectively). B. VGCC tail currents after repolarization in control, Ros, and GV-58-modified conditions. C. Novel analog structures. D. Effects of novel analogs on tail current integral.

Hypothesized access and mechanisms of action of GV-58

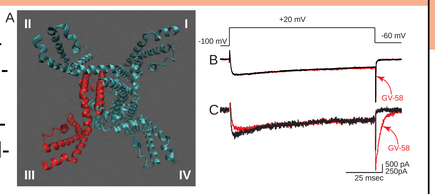


GV-58 can only access the closed state, but only modify the open state. A & B. Effects of KK-20 following rapid application either during (**A**) or before (**B**) the 200 ms voltage step used to activate Ca^{2+} current. Colored bars show time course of KK-20 application. Top traces show 200 ms voltage steps that activate currents. Bottom traces show tail currents in the absence of KK-20 (black line), after 1 sec KK20 application before and during the voltage step (dotted line), and after applying KK-20 only for 100 ms as indicated (green and red lines). **C**. When normalized to the maximum observed (100 ms step depolarization to +20 mV), very brief depolarizations (1-3 ms) only allow GV-58 to modify a significant, but smaller fraction of VGCCs. Inset: Sample tail current traces (normalized at their peaks) in response to 1, 2, and 100 ms depolarizations.

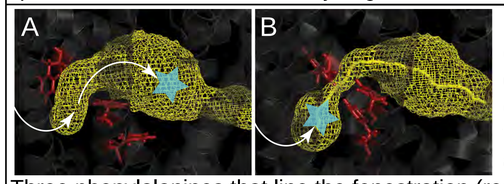


Model for access and binding of GV-58 to Cav2 VGCCs. A. GV-58 (red star) is hypothesized to partition into plasma membrane (PM) and a fenestration in VGCCs that is only accessible in the closed channel. **B**. GV-58 that is inside the fenestration is exposed to a binding site (light blue) that is only present in the open configuration. **C**. GV-58 binding stabilizes the open state (green star). **D**. Preliminary kinetic model of GV-58 modulation that fits experimental data (**E**).

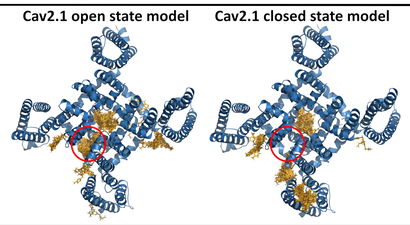
Where does GV-58 bind within Cav2 channels?



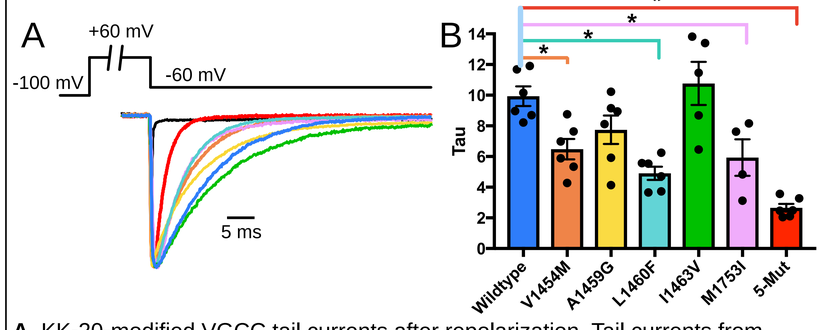
A. Top view of GV-58 binding region (red, domain III), based on channel chimera experiments. **B**. Voltage-clamp recording of Ca^{2+} current from a HEK293 cell expressing Cav1 channels (insensitive to GV-58). **C**. Voltage-clamp recording from a HEK293 cell expressing a chimeric channel in which domains I, II, and IV are from Cav1.2, and domain III is from Cav2.1 (a sensitive channel). The presence of a Cav2.1 domain III is sufficient for GV-58 to prolong deactivation of this chimeric channel. In fact, other experiments have shown that only segments S4-6 of domain III are required.



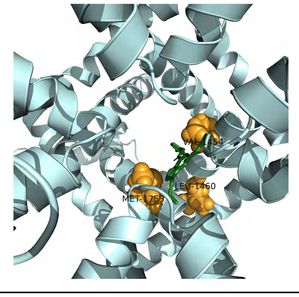
Predicted Cav2.1 fenestration (yellow) in the closed (**A**) and open (**B**) configurations. GV-58 (blue star) traverses the wider fenestration in the closed state to access the channel interior. Three phenylalanines that line the fenestration (red) are predicted to gate fenestration diameter.



Cav2.1 open and closed state homology models with predicted GV-58 binding poses shown in yellow. The region chosen for experimental evaluation is circled, showing a cluster of GV-58 poses predicted in the open state, but not in the closed state.



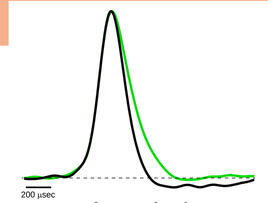
A. KK-20-modified VGCC tail currents after repolarization. Tail currents from HEK293 cells expressing each VGCC mutant (color coded as in the bar graph in **B**). **B.** Mean KK-20-modified tau of deactivation for wild type Cav2.1 and each mutant. Larger taus represent slower deactivation. Mutant channels (in the absence of KK-20) had deactivation kinetics that were not significantly different between wild type or each other (not shown). Significant differences for all comparisons were determined by one-way ANOVA with Tukey's post hoc test ($p < 0.05$).



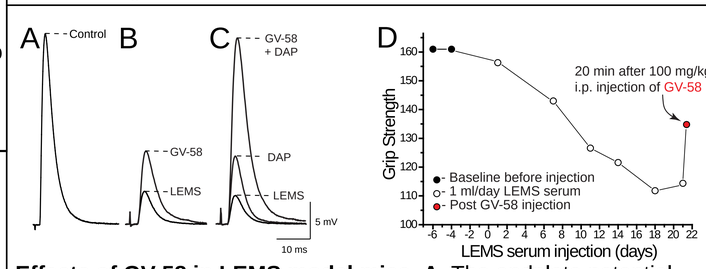
Close-up of proposed binding pocket. Yellow: Predicted binding residues whose role in mediating GV-58 analog activity was validated in mutagenesis experiments. **Green:** Predicted GV-58 binding pose that contacts these residues.

Therapeutic potential of GV-58 for treatment of neuromuscular diseases

LEMS is caused by autoimmune-mediated removal of a fraction of presynaptic VGCCs, which decreases acetylcholine release resulting in muscle weakness. There is no cure, and symptomatic treatment strategies that increase transmitter release are recommended. The recently approved potassium channel blocker 3,4-diaminopyridine, or DAP (which increases the percentage of VGCCs that are opened by a broadened AP) is only modestly effective due to dose-limiting side-effects.

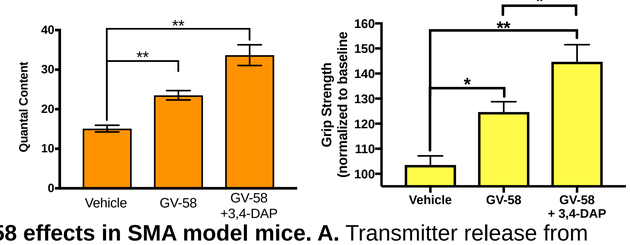


AP waveforms in the motor nerve terminal imaged using the voltage-sensitive dye BeRST-1. 1 μM DAP broadens the AP duration.



Effects of GV-58 in LEMS model mice. A. The endplate potential (EPP) in a control mouse NMJ ("Control"). **B & C.** Two EPP recordings are shown after LEMS passive transfer to mice ("LEMS"). **B.** 50 μM GV-58 doubles EPP size ("GV-58"). **C.** 1.5 μM DAP doubles EPP size ("DAP"). The synergistic activity of 1.5 μM DAP plus 50 μM GV-58 (GV-58 + DAP) results in a significantly stronger effect than DAP or GV-58 alone and returns EPP size to control levels. **D.** GV-58 effects on grip strength in a LEMS model mouse. Baseline grip strength (black circles) is reduced by daily LEMS serum injection (open circles) but is reversed 15 minutes after a GV-58 injection (red circle).

SMA is a genetic disease that results in motoneuron degeneration. Recently approved gene therapy increases motoneuron survival, but peripheral NMJ pathology persists. Therefore, additional treatment strategies that restore synaptic function would be therapeutic.



GV-58 effects in SMA model mice. A. Transmitter release from PD11-13 SMNΔ7 mouse epitrochleoanconeus NMJs (quantal content) is significantly increased by 50 μM GV-58, and by 50 μM GV-58 plus 1.5 μM DAP. **B.** PD10 SMNΔ7 mouse muscle strength (grip strength) is significantly increased by an injection of GV-58 or GV-58 plus DAP.

References

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