Preferential Inhibition of Persistent Sodium Current is an Effective Antiepileptic Drug Mechanism

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SUPPLEMENTAL INFORMATION

METHODS

Drugs and compounds

Ranolazine dihydrochloride (Sigma-Aldrich, St. Louis, MO, USA), phenytoin sodium injection, USP (Baxter Healthcare, Corp., Deerfield, IL, USA), phenytoin (Sigma-Aldrich, St. Louis, MO, USA) and GS967 (Gilead Sciences, Foster City, CA, USA) were used. A solution of ranolazine was prepared in Dulbecco's phosphate buffered saline (PBS) with pH readjusted to 7.1. Drug-free PBS was used as a vehicle control for ranolazine experiments. Phenytoin sodium was diluted in 0.5% methyl cellulose, and the vehicle control solution contained 10% ethyl alcohol, 40% propylene glycol and 50% water diluted in 0.5% methyl cellulose. The pH of phenytoin and vehicle solutions was between 7.5 and 8.0. A homogenous mixture of GS967 and Purina 5LOD chow was prepared by Research Diets, Inc. GS967 chow was formulated at concentrations of 2 mg/kg and 8 mg/kg for experiments involving *Scn2a*^{Q54} mice. A solution of GS967 was prepared in a vehicle solution containing 15% NMP (1-Methyl-2-pyrrolidinone), 10% solutol HS-15 and 75% water for MES experiments. For electrophysiological experiments, phenytoin and GS967 were prepared at stock concentrations of 50 mM and 10 mM respectively in DMSO, and diluted in the appropriate bath solution at the time of recording. The final DMSO concentration was always less than 0.02%.

In vivo pharmacology

Non-transgenic F1 wild-type (C57BL/6J x SJL/J) mice purchased from The Jackson Laboratory (Bar Harbor, ME, USA) were used to study ranolazine pharmacokinetics *in vivo*. Ranolazine (40 mg/kg) was administered as a single intraperitoneal (i.p.) injection in a volume of 10 ml/kg body weight. At selected time points (10, 20, 30 or 40 minutes), four animals per group were deeply anesthetized before collecting blood and brain samples. Blood and brain samples were also collected from the phenytoin and GS967 experimental animals. Plasma was isolated by centrifugation (9000 x g, 10 minutes). Brain tissue was homogenized in 3% sodium fluoride solution containing 1% HCl (300 mg tissue in 600 µl). Ranolazine and GS967 concentrations were measured by liquid chromatography coupled with tandem mass spectrometry as previously described (Kahlig, *et al.*, 2010). Plasma phenytoin concentration was assayed by Vanderbilt University Medical Center Core Chemistry Laboratory using fluorescence polarization immunoassay technology (COBAS INTEGRA; Roche, Basel, Switzerland).

Mossy Fiber Sprouting

Timm staining, used to detect mossy fiber sprouting, was performed as previously described (Sloviter, 1982). Mossy fiber sprouting was measured by quantifying the density of stain within the inner molecular layer of the dentate gyrus from both the suprapyramidal and infrapyramidal blades. An image of the dentate gyrus was obtained with a 10x objective from 2 sections from each mouse. NIH ImageJ was used to acquire a total of 6 density measurements from each section (12 measurements per mouse). Each measurement was normalized to the background staining within the outer molecular layer of the section. These values were averaged to obtain a single density measurement for each animal. Mossy fiber sprouting was compared among groups using one-way ANOVA followed by Tukey HSD post-hoc tests and $p < 0.05$ was considered statistically significant. Statistical analysis was conducted using STATA 12.0 (StataCorp LP, College Station, TX).

Table S1: Effect of GS967 on rat $Na_V1.2$-GAL897-881QQQ Biophysical Properties

	Voltage Dependence of Activation			Voltage Dependence of Fast Inactivation			Recovery from Fast Inactivation		
	$V_{1/2}$ (mV)	**k (mV)**	***n***	**$V_{1/2}$ (mV)**	**k (mV)**	***n***	**τ_f (ms)**	**τ_s (ms)**	***n***
Control	-15.2 ± 0.8	8.5 ± 0.2	11	-67.5 ± 1.4	-5.4 ± 0.1	9	7.3 ± 0.5 (84 ± 2%)	572.1 ± 81.6 (17 ± 4%)	14
GS967	-18.1 ± 0.9*	8.6 ± 0.2	11	-71.1 ± 1.6*	-5.5 ± 0.2	9	15.2 ± 3.*2 (40 ± 5%*)	78.9 ± 8.5* (56 ± 4%*)	15

* indicates $p < 0.05$ compared to control conditions

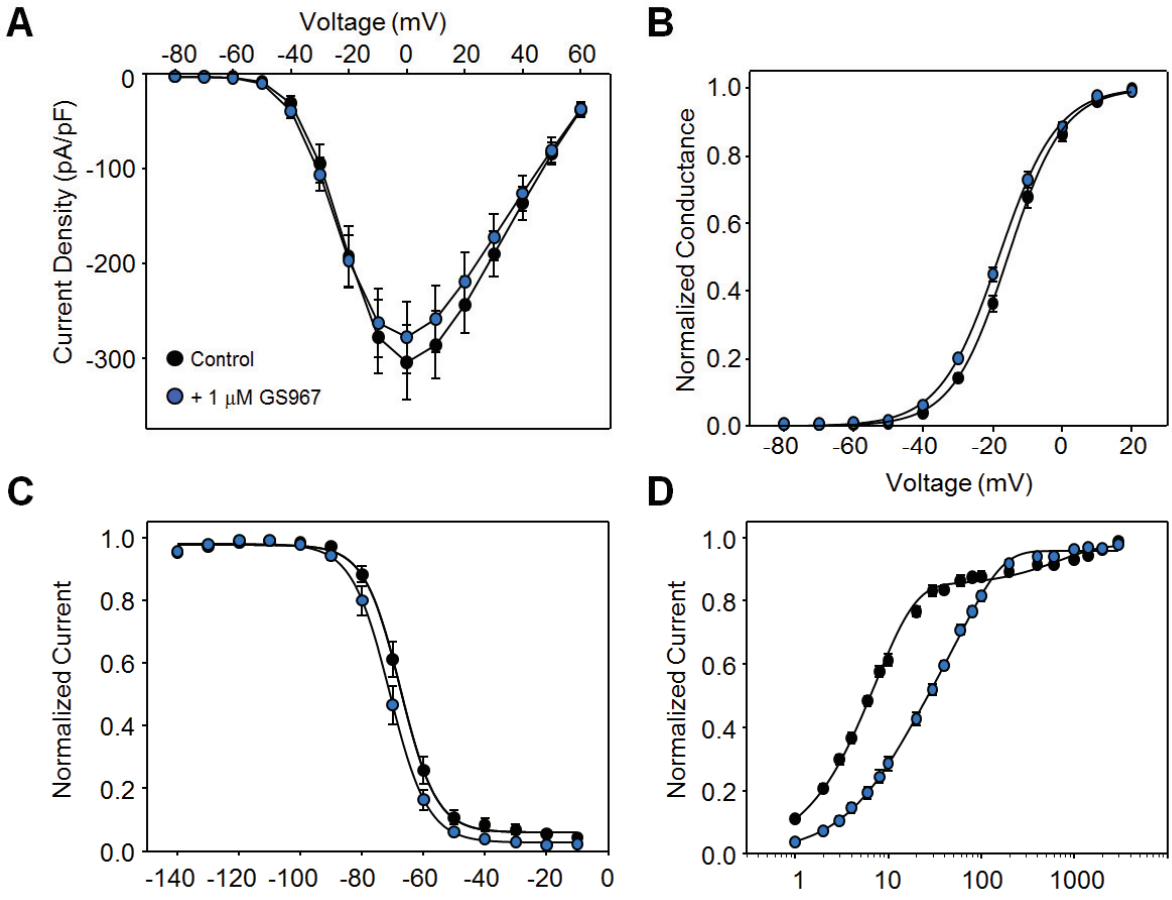

Figure S1 - Effect of GS967 on rat $Na_V1.2$-GAL879-881QQQ biophysical properties. **(A)** Peak current density elicited by test pulses to various membrane potentials and normalized to cell capacitance. **(B)** Voltage dependence of channel activation measured between −80 to +20mV **(C)** Voltage dependence of inactivation measured following a 100 ms inactivating prepulse ranging from −140 to −10 mV. **(D)** Time-dependent recovery from inactivation assessed with a 100 ms inactivating prepulse to −10 mV. Black symbols represent data recorded under control conditions while blue symbols are data recorded in the presence of 1 μM GS967. Data are represented as mean ± S.E.M., with n = 9 – 11 cells.

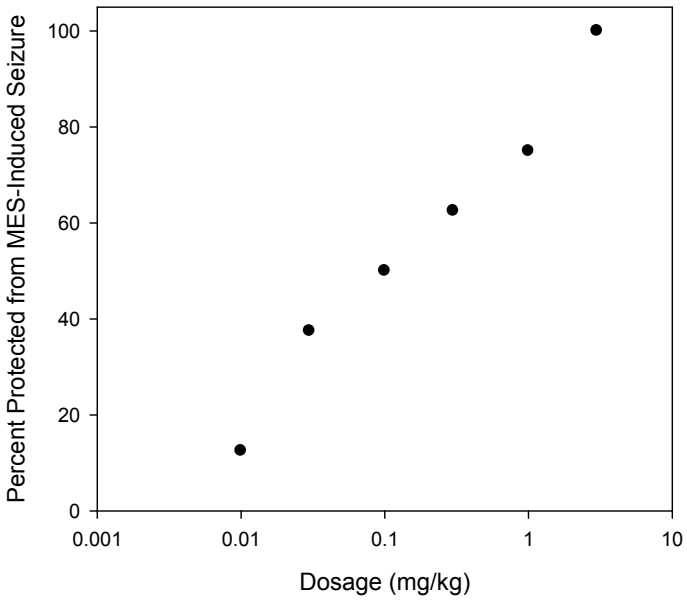

Figure S2 - GS967 protects against MES-induced seizures. Dose-response curve for seizure protection by GS967. Data are shown as percentage of animals protected from seizure at a given dose of drug, with n = 5-8 animals per dose.