Functional effects of the M-current activator BHV-7000 on 50 epilepsy-associated *KCNQ2* variants

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ABSTRACT

Rationale: *KCNQ2* and *KCNQ3* encode potassium channel subunits (Kv7.2, Kv7.3), which co-assemble to generate neuronal M-current that regulates neuronal excitability. *KCNQ2* pathogenic variants identified in children with self-limited familial neonatal epilepsy (SeLFE) and developmental and epileptic encephalopathy (DEE) most often exhibit loss-of-function (LoF). Potentiation of M-current with agents such as BHV-7000 (formally KB-3061) is a potential therapy for these conditions. We determined the effects of BHV-7000 on 50 loss-of-function *KCNQ2* variants (10 SeLFNE-associated and 40 DEE-associated)

Methods: We expressed Kv7.2 with Kv7.3 in Chinese hamster ovary (CHO) cells and recorded whole-cell currents using automated planar patch clamp first in the absence then presence of vehicle (DMSO) or BHV-7000. CHO-K1 cells stably expressing WT Kv7.3 were transfected with an equal mixture of wild type (WT) and variant KCNQ2 to generate heteromultimeric channel complexes that recapitulated the heterozygous state without detectable expression of Kv7.2 homotetramers. BHV-7000 effects were assessed at 0.3, 1.0, 3.0, 10 and 30 μM. Specific channel activities were determined by applying the M-current blocker XE-991 (10 μM) at the end of experiments; only XE-991-sensitive currents were analyzed.

Results: We measured the effect of BHV-7000 on current density measured at -30 mV, which we assert is within the physiological relevant range of neuronal M-current. At 1 μM exposure, BHV-7000 restored current density for most variants to ≥ 76% of WT channels measured in the absence of drug. Current density for two DEE-associated variants (E140Q, D282N) was restored to approximately 65% of WT levels at this concentration, but current density of both variants was boosted 90-150% at higher concentrations (D282N = 90% at 3 μM; E140Q = 150% at 10 μM). At 3 μM exposure, BHV-7000 induced hyperpolarizing shifts in the voltage-dependence of activation for 42 variants to a degree similar to WT channels (delta V½ of averaged WT channels was -17.5 ± 4.0 mV [mean ± stdev, n =12]). Six variants exhibited smaller degrees of hyperpolarization and 2 showed larger hyperpolarization. The averaged EC_{50} values for BHV-7000 induced shift in V½ and current density increase measured at -30 mV in WT channels were 1.1 ± 0.9 μM and 3.5 ± 0.9 μM, respectively (mean ± stdev, n =12). For the majority of tested variants, the calculated EC_{50} values fell within 1 stdev of the WT average for current density and shift in activation V½.

Conclusions: BHV-7000, a selective M-current activator restored current density in all tested pathogenic *KCNQ2* variants. For most of the tested variants, current density was restored to near WT levels with 1 μM BHV-7000. The calculated BHV-7000 EC_{50} values were similar to WT for the majority of the variants tested. These findings support the potential therapeutic value of BHV-7000 in *KCNQ2*-related epilepsy associated with a wide range of variants.

Background

KCNQ2 pathogenic variants identified in children with developmental and epileptic encephalopathy (DEE) most often exhibit loss-of-function with dominant-negative effects. Pharmacological potentiation of M-current is a potential therapy for this condition. Our previous results (Vanoye et al,2022, JCI Insight) demonstrated genotype-dependent differences in the response of *KCNQ2* variants to retigabine, a proposed precision therapy for *KCNQ2* DEE. In this study we investigated whether the investigational agent BHV-7000 (BHV; formally KB-3061) exhibits genotype-dependent differences.

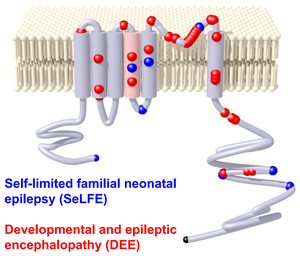
Methods

Cell Culture: Chinese hamster ovary cells (CHO-K1) stably expressing human KCNQ3-WT (CHO-Q3) were grown in F-12 nutrient mixture medium supplemented with fetal bovine serum, penicillin and streptomycin with hygromycin selection at 37°C in 5% CO_2.

Molecular Biology: Variants were introduced into human KCNQ2 using Quikchange mutagenesis (Agilent technologies). KCNQ2 variants were expressed from plasmid pIRES2_KCNQ2_EGFP, whereas KCNQ2-WT was expressed from plasmid pIRES2_KCNQ2_CyOFP. These plasmids included green or orange fluorescent proteins, respectively, as transfection markers. The KCNQ2 reading frame of all constructs was sequenced completely.

Electroporation: Using the MaxCyte STX system, WT plus variant KCNQ2 cDNAs were transiently co-transfected into CHO_KCNQ3 cells to resemble the heterozygous state. Transfection efficiency was evaluated by flow cytometry using a 488 nm laser and filters for green fluorescence (FITC, KCNQ2_variants coupled to EGFP expression), and orange fluorescence (PEA, KCNQ2_WT couple to CyOFP expression).

Electrophysiology: Automated planar patch clamp recording was performed on the Nanion SyncroPatch 384 platform using 4X S-Type chips. External solution contained (in mM): 140 NaCl, 4 KCl, 2.0 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 5 glucose, pH 7.4. The composition of the internal solution was (in mM): 60 KF, 50 KCl, 10 NaCl, 10 EGTA, 10 HEPES, 5 mM MgATP, pH 7.2. BHV-7000 (Biohaven) and Retigabine (SIGMA) were added from 60 and 50 mM (respectively) stocks solutions dissolved in DMSO. DMSO volume was constant for all concentrations for each compound (0-30 μM; 0.5 or 0.6 μl/ml). Whole-cell currents were recorded at 22°C from a holding potential of -80 mV using 1000 ms depolarizing pulses from -80 to +40 mV (in 10 mV steps), followed by a 250 ms step to 0 mV to analyze tail currents. Voltage-dependence of activation values were derived from tail currents normalized to maximal tail current amplitude and expressed as a function of the preceding voltages. Data were then fit to a Boltzmann function: *I(V) = Bottom + (Top-Bottom) / (1 + exp((V½ -V)/ slope))*. EC_{50} values were calculated with the equation *Y = Bottom + X * (Top-Bottom)/ (EC_{50} + X)*. Current and voltage data are presented as mean ± 95% Confidence Intervals, EC_{50} data are shown as mean ± SEM. Number of cells is given in the figure legends. Total number of cells analyzed = 11946: wild type = 1287, variant = 10659.,



S113F	A265V	Y284D	R333Q
E130K	W269S	Y284H	R333W
E140Q	T276I	Y284N	P335A
R144Q	T276P	G290D	P335L
A193D	T277I	R291G	V543M
L203P	T278I	L292P_L293	R560W
R207W	G279C	F304S	D563N
D212Y	G279S	F305del	R581G
H228R	Y280H	F305L	R581Q
H228Y	G281W	A306P	R588S
L243F	D282H	A306T	L637R
S247L	D282H	A306V	
G256R	Y284C	H328N	

Figure 1– Variants analyzed in this study and location in the KCNQ2 protein.

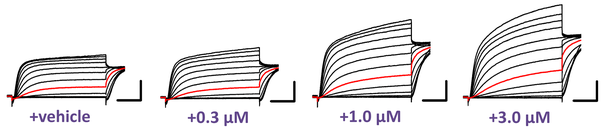


Figure 2 – Wild type channels recorded under various BHV-7000 concentrations.

Whole-cell currents recorded in the presence of XE-991 were digitally subtracted from the +compound data and normalized to membrane capacitance. Only the XE-991-sensitive currents were analyzed. Whole-cell currents were measured at the end (998 ms) of a 1000 ms long voltage step. **Red** lines indicate currents recorded at -30 mV. Scale bars are 250 ms (horizontal) and 25 pA/pF (vertical).

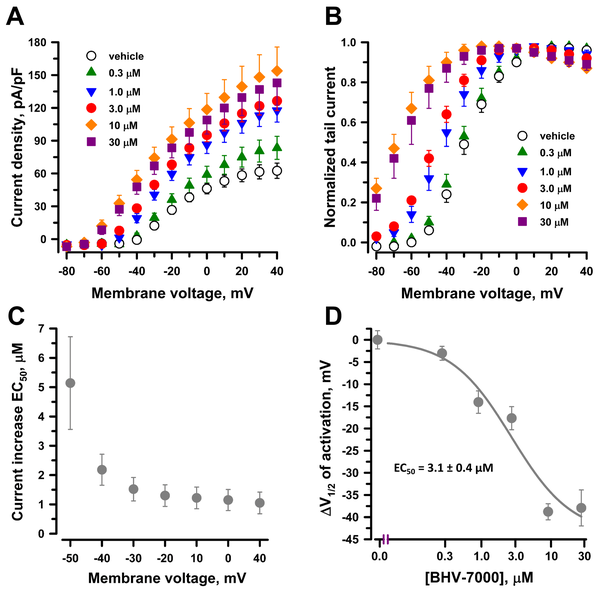


Figure 3 – Effects of BHV-7000 on KCNQ2/KCNQ3 channels.

Current-voltage relationships **(A)** and voltage-dependence of activation curves **(B)** for WT KCNQ2 / KCNQ3 channels exposed to 0-30 μM BHV-7000. **C.** Half maximal effective concentration (EC_{50}) values calculated for current increase at various membrane potentials. **D.** EC_{50} values calculated for hyperpolarizing shifts in activation V½ (142-234 cells per condition).

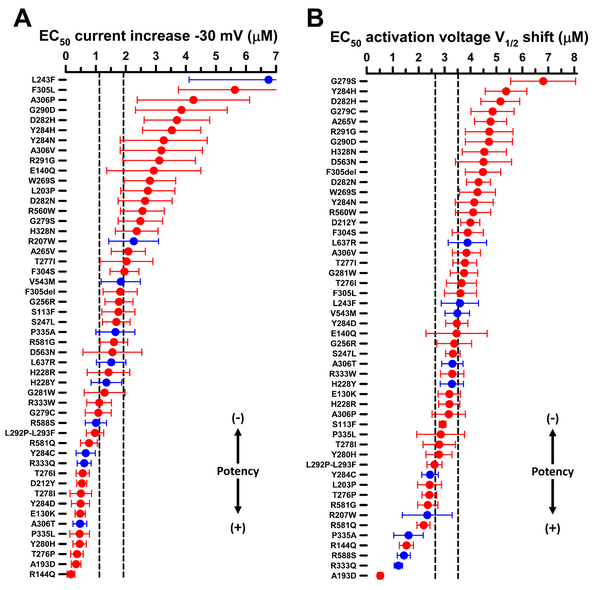


Figure 4 – Potency of BHV-7000 differs among epilepsy-associated heterozygous variant KCNQ2 / KCNQ3 channels.

Waterfall plots showing half maximal effective concentration (EC_{50}) values calculated for increase in current density recorded at -30 mV (**A**) and hyperpolarizing shifts in activation V½ (**B**). EC_{50} and V½ values were calculated as described in Fig. 3 legend. **Blue** symbols denote Self-limited familial neonatal epilepsy (SelFE), **red** symbols indicate developmental and epileptic encephalopathy (DEE). Dashed lines indicate mean ± SEM values for wild type KCNQ2 / KCNQ3 currents (57-156 cells per variant).

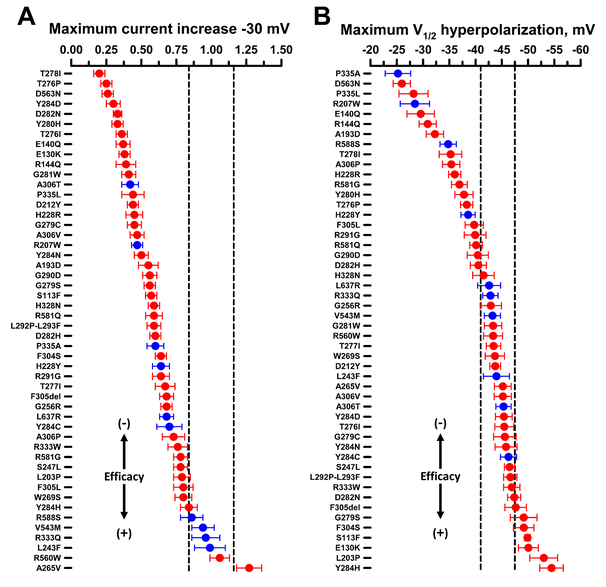


Figure 5 – Efficacy of BHV-7000 differs among epilepsy-associated heterozygous variant KCNQ2 / KCNQ3 channels.

Waterfall plots depicting maximum current density increase recorded at -30 mV (**A**) and activation V½ hyperpolarization (**B**). Maximum values were calculated with the equation *Y = Bottom + X * (Top-Bottom)/ (EC_{50} + X)*, as the difference between *Top* and *Bottom*. **Blue** symbols = SeLFE, **red** symbols = DEE. Dashed lines indicate mean ± 95% CI values for wild type KCNQ2 / KCNQ3 channels (57-156 cells per variant).

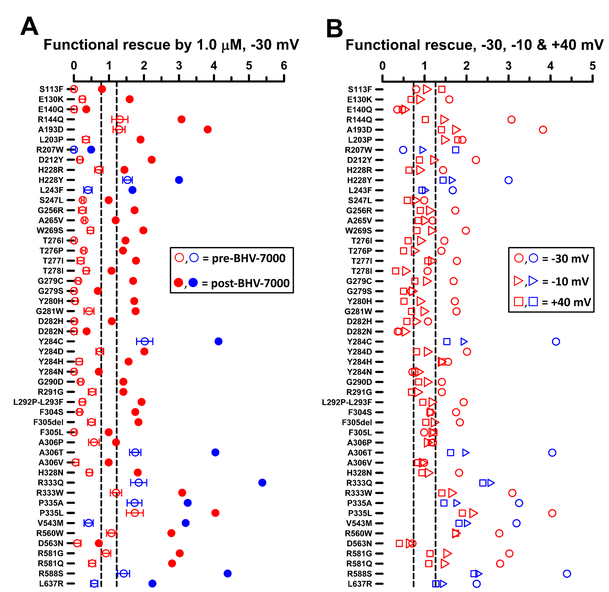


Figure 6 – Functional rescue of M-current by BHV-7000.

A. Current density from variant channels measured at -30 mV after exposure to 1 μM BHV-7000 normalized to vehicle-treated WT currents recorded at -30 mV. Empty symbols indicate pre-compound data, filled symbols are values after compound addition. **B.** Current density from variant channels measured at -30 (○,○), -10 (▷,▷) or +40 (□,□) mV after exposure to 1 μM BHV-7000 normalized to currents recorded from vehicle-treated WT channels at the respective potential. **Blue** symbols = SeLFE, **red** symbols = DEE. Dashed lines indicate mean ± 95% CI values for WT vehicle-treated channels. (14-28 cells per variant).

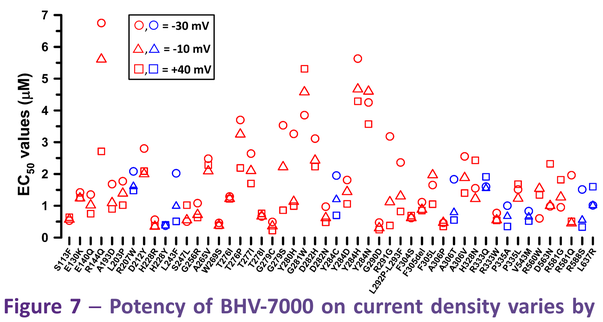


Figure 7 – Potency of BHV-7000 on current density varies by membrane potential.

EC_{50} values calculated for increase in current density recorded at -30 (○,○), -10 (△,△) or +40 (□,□) mV after exposure to 1 μM BHV-7000. **Blue** symbols = SeLFE, **red** symbols DEE (57-156 cells per variant).

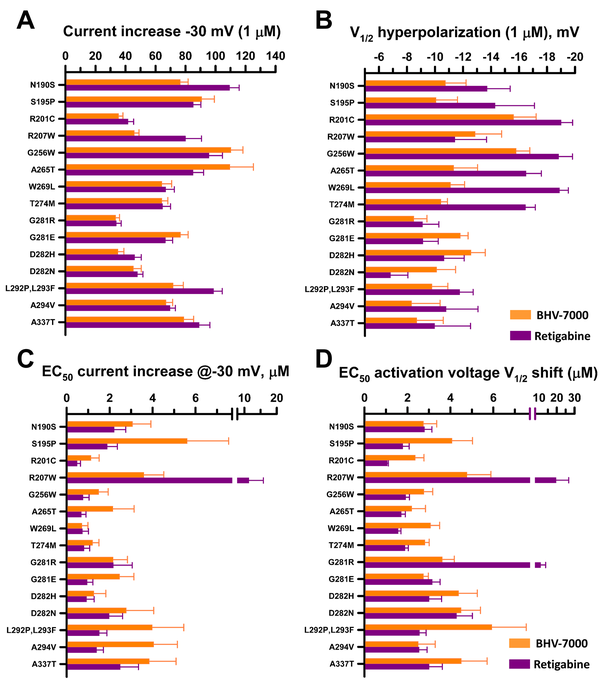


Figure 8 – Comparison of BHV-7000 and retigabine effects on KCNQ2 variants from patients treated with retigabine.

Specific KCNQ2 variants were studied because of reported responses to retigabine. Current density increase recorded at -30 mV (**A**) and V½ hyperpolarization shift (**B**) from KCNQ2 variant channels expressed as heterozygous exposed to 1 μM BHV-7000 or retigabine. EC_{50} values calculated for increase in current density recorded at -30 mV (**C**) and hyperpolarizing shifts in activation V½ (**D**). (83-190 cells per condition).

Summary

1. Calculated BHV-7000 EC_{50} values were similar to WT for the majority of the KCNQ2 variants tested, while the efficacy varied among the variants.
2. For most tested variants, current density was restored (e.g., rescued) to WT-like levels with 1 μM BHV-7000 at -30 mV.
3. Membrane potential influences the level of BHV-7000 rescue and potency on KCNQ2 current.
4. For most variants tested, BHV-7000 and retigabine exhibit similar potency on current density increase and hyperpolarization of activation $V_{1/2}$.
5. For most variants BHV-7000 and retigabine increase current density to similar levels (1 μM, -30 mV), with BHV-7000 causing a smaller shift in activation $V_{1/2}$.

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