POSTER
3.466

Opakalim (BHV-7000) Potentiates the Activity of Kv7.2/Kv7.3 Channels Containing a Single Copy of the Kv7.2 DEE-causing Mutation, G281E

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INTRODUCTION

- Five Kv7 subunits (Kv7.1– Kv7.5) are encoded by *KCNQ1–KCNQ5* genes.
- Kv7 subunits can assemble as homotetrameric or heterotetrameric (e.g., Kv7.2/Kv7.3) voltage-gated potassium (K^+) channels.
- Kv7.2/Kv7.3 channels in neurons play a critical role in helping maintain excitatory/inhibitory balance.
- *KCNQ2* and *KCNQ3* mutations cause a spectrum of Developmental Epileptic Encephalopathies (DEE) with a broad diversity of symptoms and degree of severity.
- It has been suggested that the degree of severity of a given mutation on channel activity correlates with the severity of DEE symptoms.
- Small-molecule modulators of Kv7 channels like the Kv7-selective activator, opakalim (BHV-7000), may provide therapeutic benefit to individuals with *KCNQ* mutation-driven DEEs.
- Compassionate use treatment with opakalim was requested for a 9-year-old male patient with severe, Kv7.2 mutant (G218E)-driven DEE.
- The effects of Kv7 activators have been studied with many Kv7.2 mutant-driven DEE[1], however, the effects of Kv7 activators on the Kv7.2 G281E mutation identified in the patient have yet to be investigated.
- This patient is now being successfully treated with opakalim after treatment with a first-generation Kv7 activator was discontinued. Prior attempts to discontinue the first-generation Kv7 activator resulted in status epilepticus.
- Clinical data regarding this patient are presented on poster **3.469**.
- In addition to treatment of patients with mutant Kv7-driven DEE, opakalim is currently in late-stage development for treatment of focal epilepsy.

OBJECTIVE

The objectives of the studies described herein are to:

- Determine whether the G281E mutation of one or both Kv7.2 subunits in a Kv7.2/7.3 heteromer produce measurable currents when expressed in HEK-293 cells.
- If so, how currents arising from G281E mutant-containing Kv7 channels compare to wild-type (WT) Kv7.2/Kv7.3 currents in terms of channel activity and pharmacological properties.

METHODS

- As a reference control, expression constructs were made for WT Kv7.2 and WT Kv7.3 subunits on separate plasmids.
- To control subunit stoichiometry, Kv7.2 and Kv7.3 subunits were concatenated with an eight-glycine (8-G) linker bridging the carboxyl and amino termini of one subunit to another[2] as shown in **Figure 2**.
- Lipofectamine 3000 was used to transfect HEK-293 cells with the Kv7 expression constructs plus a plasmid encoding GFP. Transfected cells were incubated for 48 h at 37ºC in a cell-culture incubator.
- For manual voltage-clamp experiments, GFP-expressing cells were identified using fluorescence microscopy. Voltage-clamp recordings were made in whole-cell configuration using an Axon MultiClamp 700B amplifier.
- The voltage-clamp protocol used is depicted in **Figure 3**. Cells were held at –90 mV and 1500-ms steps were applied from –110 mV to +30 mV in 10-mV increments each followed by a 400-ms step to 0 mV. Steady-state current amplitudes were measured at +30 mV. Voltage-dependence of activation (V1/2) was determined from tail currents measure at 0 mV and fit to a Boltzmann equation.
- For pharmacology experiments, opakalim or ezogabine were administered to Kv7-expressing cells via local perfusion.

Figure 1. Subunit Composition

A: A diagram of a Kv7.2 subunit depicting the location of the DEE-causing G281E mutation. **B:** Tetrameric Kv7 subunit combinations where blue represents WT Kv7.2, green represents WT Kv7.3 and fuchsia represents G281E mutant Kv7.2.

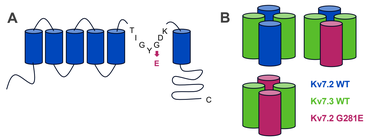


Figure 2. Kv7 Expression Constructs

Diagram of the different gene constructs used in this study. **A:** Kv7.2 and Kv7.3 subunits were expressed from separate, co-transfected plasmids. **B – D:** Kv7.2 and Kv7.3 subunits were concatenated with an eight-glycine linker between subunits.

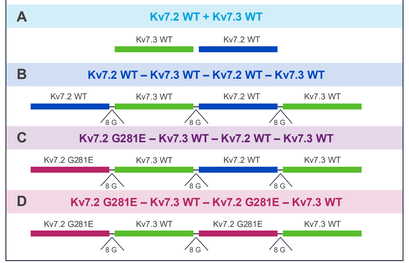
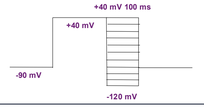


Figure 3. Voltage Protocol

Voltage-clamp protocol for measurements in **Figures 4** and **5**.



RESULTS

- Currents arising from co-expressed, unconcatenated WT as well as concatenated WT Kv7.2/ Kv7.3 were indistinguishable based on whole-cell current amplitude (**Figure 4A** and **4B**).
- Currents arising from concatenated Kv7.2/Kv7.3 channels containing a single G281E mutant Kv7.2 subunit averaged 8-fold smaller than WT Kv7.2/Kv7.3 currents (**Figure 4A** and **4C**).
- The concatenated Kv7.2/Kv7.3 construct bearing two G182E mutant Kv7.2 subunits failed to generate currents distinguishable from untransfected HEK-293 cells (**Figure 4D**).
- Both ezogabine and opakalim potentiated unconcatenated WT Kv7.2/Kv7.3, concatenated WT Kv7.2/Kv7.3, and concatenated Kv7.2/Kv7.3 channels bearing one mutant Kv7.2 G281E subunit (**Figure 5A, 5B, 5C**).
- The effect on half-maximal voltage of activation (ΔV1/2), a key feature of opakalim's and ezogabine's Kv7 activation, was similar for unconcatenated WT Kv7.2/Kv7.3, concatenated WT Kv7.2/Kv7.3, and concatenated Kv7.2/Kv7.3 channels with one mutant Kv7.2 G281E subunit (insets in **Figure 5A, 5B,** and **5C**).

Figure 4. Channel activity of WT and Mutant Kv7.2/Kv7.3 Channels

Voltage-clamp current traces from Kv7 constructs expressed in HEK-293 cells**. A:** Unconcatenated, co-express WT Kv7.2 + WT Kv7.3 **B:** Concatenated WT Kv7.2-Kv7.3 **C:** Concatenated Kv7.2-Kv7.3 with a single mutant Kv7.2 G281E **D:** Concatenated Kv7.2-Kv7.2 with two mutant Kv7.2 G281E. Data shown are representative of N ≥ 8 individual cells/condition.

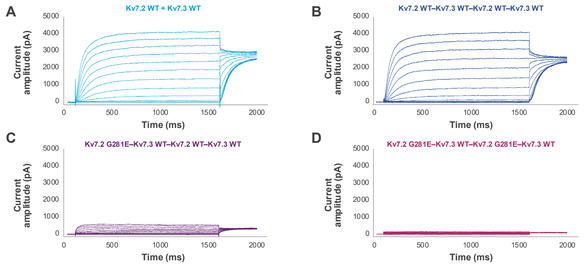
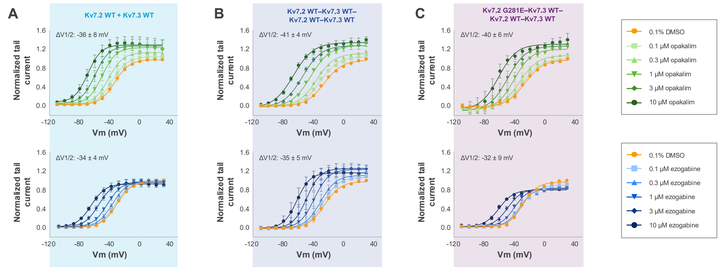


Figure 5. Effects of Opakalim and Ezogabine on WT and Mutant Kv7.2/Kv7.3 Channels

Concentration-dependent effects on Kv7.2/Kv7.3 currents resulting from treating cells with either opakalim (upper graphs) or ezogabine (lower graphs). **A:** Unconcatenated, co-express WT Kv7.2 + WT Kv7.3 **B:** Concatenated WT Kv7.2-Kv7.3 **C:** Concatenated Kv7.2-Kv7.2 with a single mutant Kv7.2 G281E. Insets show changes in ΔV1/2 ± SEM (10 μM drug). Data are normalized to vehicle control (0.1% DMSO) for each construct.



CONCLUSIONS

- The G281E mutation in Kv7.2 is one of the two glycine residues in the canonical K^+ channel pore-loop GYG motif and results in a severe loss of function measured in vitro, likely producing pathologically reduced Kv7.2/Kv7.3 currents in the patient bearing the mutation resulting in the observed severe DEE symptoms.
- Opakalim and ezogabine can activate Kv7.2/Kv7.3 channels containing a single copy of the mutant Kv7.2 G281E subunit to a similar relative degree as WT Kv7.2/Kv7.3 channels. These findings are consistent with our previous findings with Kv7.2/Kv7.3 channels containing different Kv7.2 mutations[1].
- This in vitro approach provides a translational method to better evaluate the effects of Kv7.2 mutations on Kv7.2/Kv7.3 channel activity and pharmacology to help inform treatment of other DEE patients.
- Given that a patient with a severe DEE, bearing a Kv7.2 mutation resulting in a severe loss of channel activity, is being successfully treated with opakalim, other patients with mutant Kv7-channel-driven DEEs will likely benefit from opakalim treatment.

American Epilepsy Society (AES) Annual Meeting
December 5–9, 2025 | Atlanta, GA

DISCLOSURES: All authors are employed by and hold stock/stock options in Biohaven Pharmaceuticals. This research was funded by Biohaven Pharmaceuticals.

REFERENCES:

1. Vanoye CG, Desai RR, Dworetzky S, George AL. Functional effects of the M-current activator BHV-7000 on 50 epilepsy-associated *KCNQ2* variants. Poster presented at AES December 6-10, 2024. Available from: https://biohaven.b-cdn.net/wp-content/uploads/AES-2024-poster-BHV-7000-effects-on-KCNQ2-variants.pdf

2. Ray S, Stampf JL, Kudlacek O, et al. A triple cysteine motif as major determinant of the modulation of neuronal KV7 channels by the paracetamol metabolite N-acetyl-p-benzo quinone imine. Br J Pharmacol. 2024;181(16):2851-2868. doi:10.1111/bph.16380

3. Wilenkin B, Burris KD, Eastwood BJ, Sher E, Williams AC, Priest BT. Development of an Electrophysiological Assay for Kv7 Modulators on IonWorks Barracuda. Assay Drug Dev Technol. 2019;17(7):310-321. doi:10.1089/adt.2019.942

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