KB-3061 Is a Potent Activator of Wild Type KCNQ Channels and Restores Current to KCNQ2 Encephalopathy Variants In Vitro

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Background

Heterozygous KCNQ2 de novo variants (DNVs) are a cause of neonatal-onset developmental and epileptic encephalopathy (Q2-DEE). Most Q2-DEE variants are missense, and are clustered in hotspots that are domains for key protein functions, namely voltage sensing, trans-membrane ion flow, and sites regulating PIP2 and calmodulin binding.[1] Under voltage-clamp, many Q2-DEE DNVs exert dominant-negative effects when co-expressed with wild type (wt) subunits to mimic heterozygosity. Ezogabine (EZO) increases currents through channels containing mutated subunits in vitro and has been used in Q2-DEE patients. Novel small molecules acting as potent neuronal KCNQ channel openers are candidate precision medicines for Q2-DEE.

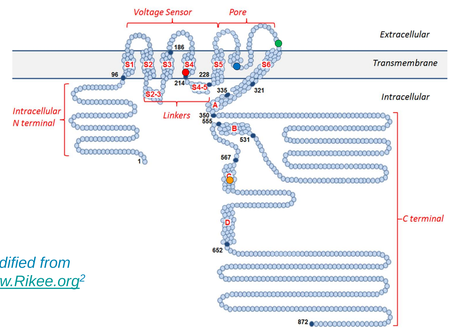
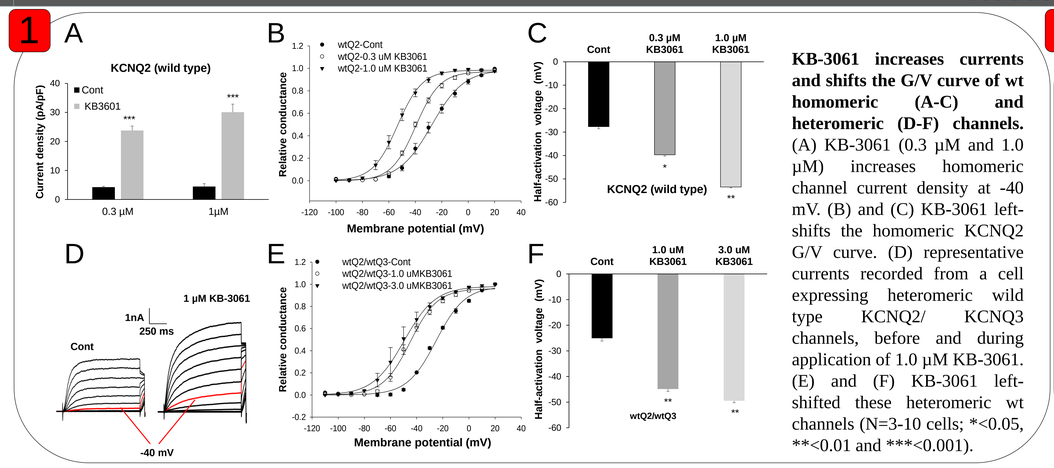
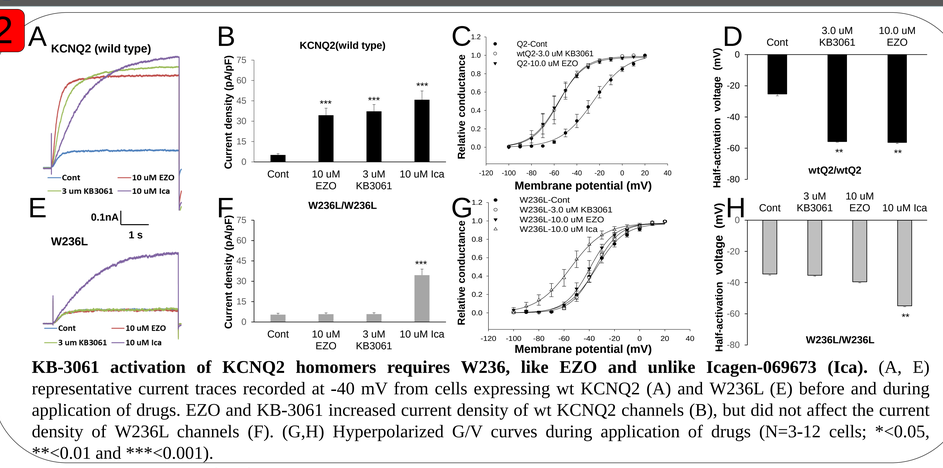


Fig.1 KCNQ2 subunit membrane folding pattern showing locations of 4 recurrent pathogenic *KCNQ2* DEE variants. R210H is in S4 of the voltage-sensor, T274M is in the pore helix, A294V is at the border of the pore loop and S6, and R581Q is in the intracellular helix C.

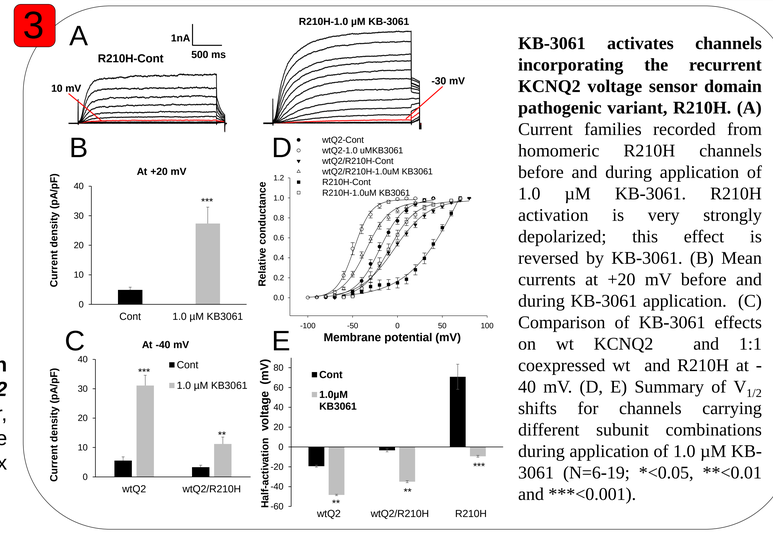
Results



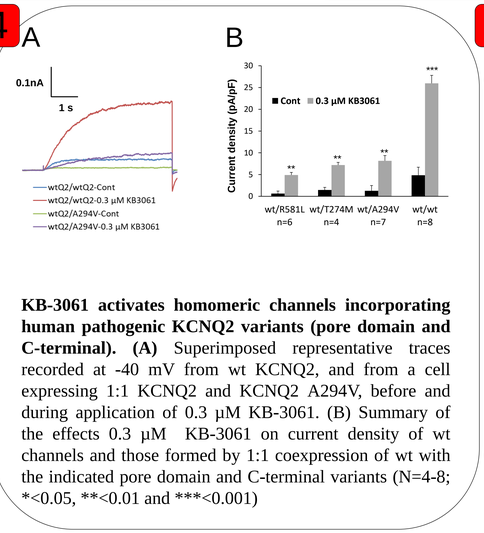
KB-3061 increases currents and shifts the G/V curve of wt homomeric (A-C) and heteromeric (D-F) channels. (A) KB-3061 (0.3 µM and 1.0 µM) increases homomeric channel current density at -40 mV. (B) and (C) KB-3061 left-shifts the homomeric KCNQ2 G/V curve. (D) representative currents recorded from a cell expressing heteromeric wild type KCNQ2/ KCNQ3 channels, before and during application of 1.0 µM KB-3061. (E) and (F) KB-3061 left-shifted these heteromeric wt channels (N=3-10 cells; *<0.05, **<0.01 and ***<0.001).



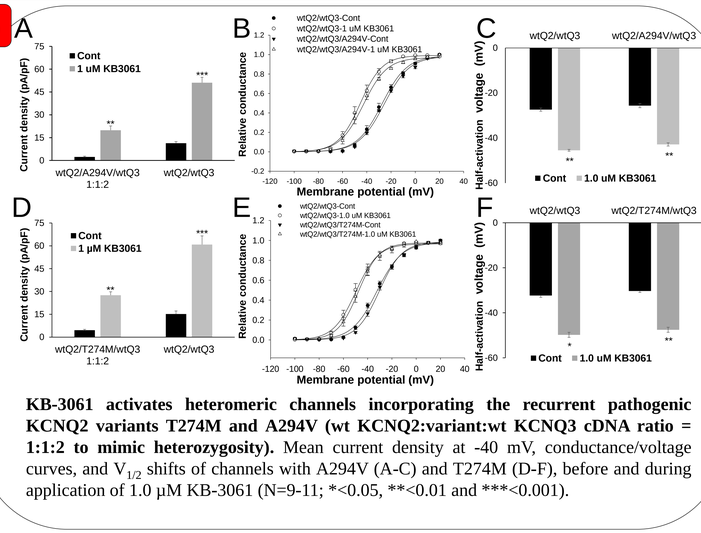
KB-3061 activation of KCNQ2 homomers requires W236, like EZO and unlike Icagen-069673 (Ica). (A, E) representative current traces recorded at -40 mV from cells expressing wt KCNQ2 (A) and W236L (E) before and during application of drugs. EZO and KB-3061 increased current density of wt KCNQ2 channels (B), but did not affect the current density of W236L channels (F). (G,H) Hyperpolarized G/V curves during application of drugs (N=3-12 cells; *<0.05, **<0.01 and ***<0.001).



KB-3061 activates channels incorporating the recurrent KCNQ2 voltage sensor domain pathogenic variant, R210H. (A) Current families recorded from homomeric R210H channels before and during application of 1.0 µM KB-3061. R210H activation is very strongly depolarized; this effect is reversed by KB-3061. (B) Mean currents at +20 mV before and during KB-3061 application. (C) Comparison of KB-3061 effects on wt KCNQ2 and 1:1 coexpressed wt and R210H at -40 mV. (D, E) Summary of $V_{1/2}$ shifts for channels carrying different subunit combinations during application of 1.0 µM KB-3061 (N=6-19; *<0.05, **<0.01 and ***<0.001).



KB-3061 activates homomeric channels incorporating human pathogenic KCNQ2 variants (pore domain and C-terminal). (A) Superimposed representative traces recorded at -40 mV from wt KCNQ2, and from a cell expressing 1:1 KCNQ2 and KCNQ2 A294V, before and during application of 0.3 µM KB-3061. (B) Summary of the effects 0.3 µM KB-3061 on current density of wt channels and those formed by 1:1 coexpression of wt with the indicated pore domain and C-terminal variants (N=4-8; *<0.05, **<0.01 and ***<0.001)



KB-3061 activates heteromeric channels incorporating the recurrent pathogenic KCNQ2 variants T274M and A294V (wt KCNQ2:variant:wt KCNQ3 cDNA ratio = 1:1:2 to mimic heterozygosity). Mean current density at -40 mV, conductance/voltage curves, and $V_{1/2}$ shifts of channels with A294V (A-C) and T274M (D-F), before and during application of 1.0 µM KB-3061 (N=9-11; *<0.05, **<0.01 and ***<0.001).

Methods

Four highly recurrent Q2-DEE missense variants (Fig. 1), R210H (S4), T274M (pore helix), A294V (pore loop), and R581Q/L (helix C), were introduced into KCNQ2 cDNA by QuikChange site-directed mutagenesis. Using lipid-mediated transfection, plasmids including the pathogenic variants were co-expressed with wt KCNQ2 or KCNQ2 and KCNQ3 subunits in Chinese hamster ovary (CHO) cells. The KCNQ2 S5 helix mutation W236L, which abolishes EZO modulation, was used to characterize KB-3061 binding. Whole cell voltage-clamp protocols were used to measure gating and conductance of wt channels and mutated subunit containing channels, before, during and after KB-3061 application via focal pipette perfusion.

Conclusions

KB-3061 activates KCNQ2-containing homomers and KCNQ2/3 heteromers by shifting activation and increasing maximal current. Such KCNQ2 modulation requires the pore domain residue W236. Variants from three different Q2-DEE mutational hotspots exhibited strong dominant-negative current suppression under conditions mimicking heterozygosity. KB-3061 (0.3 – 1 µM) augmented currents in cells expressing each of these variants, increasing currents at -40 mV to wt levels. These results suggest potential for benefit in Q2-DEE.

References

1. Millichap JJ, Park K et al 2016 KCNQ2 encephalopathy: Features, mutational hot spots, and ezogabine treatment of 11 patients. Neuro Genet. 2(5):e96.
2. The RIKEE Project. www.rikee.org/

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