A High Throughput Selection Strategy in Yeast to Study TASK-3 Potassium Channel Interactions with the Breathing Stimulant Drug PKTHPP

Authors: Anita Luethy, MD[1,2] Joseph F. Cotten, MD, PhD[1]

[1] Massachusetts General Hospital Department of Anesthesia, Critical Care, and Pain Medicine, Boston, MA, USA.
[2] Department of Anesthesia, Kantonsspital Aarau, Aarau, Switzerland.

Background: TASK-3 tandem pore potassium channels provide a constitutive potassium conductance, which regulates neuronal excitability, and that may have an important role in breathing regulation. PKTHPP is a potent inhibitor of TASK potassium channels and is an effective breathing stimulant in rodents (1). The potassium sensitive trk1Δtrk2Δ *S. cerevisae* yeast strain has been developed, previously, as a model system for studying potassium channel function (2). Additionally, growth competition experiments in yeast, combined with next generation DNA sequencing, is a new method for large scale mutational protein analysis (3).

Hypotheses: We hypothesized that PKTHPP binds in the TASK-3 channel pore. Furthermore, we hypothesized that high throughput selection in trk1Δtrk2Δ *S. cerevisae* yeast may identify TASK-3 amino acid residues in the pore essential PKTHPP binding. METHODS: To test our hypotheses, we prepared a library of randomly mutagenized TASK-3 cDNA, targeting the pore amino acid residues 120 to 128 for mutagenesis (180 possible missense mutants). We transformed this library of mutant TASK-3 cDNAs into trk1Δtrk2Δ *S. cerevisae* yeast and cultured them in low potassium media in the presence of PKTHPP (10 microM) or DMSO (vehicle only). The plasmid library cDNA was recovered from the yeast just before and just after growth/selection with DMSO/PKTHPP and TASK-3 sequence was analyzed by next generation sequencing (Illumina MiSeq platform). To quantify selection, we calculated an enrichment ratio for each TASK-3 pore mutant: [(frequency (in %) after PKTHPP growth/ frequency before PKTHPP growth)/(frequency after DMSO growth/ frequency before DMSO growth)]. PKTHPP selected for several mutations particularly at TASK-3 residue-122 with the top selected mutants being L122D, L122Q, L122K, L122E, L122Y, L122A, L122N, L122C and L128H with enrichment ratios of 343, 55, 45, 35, 28, 27, 26, 13 and 11, respectively. There was also a significant selection against specific mutations with the top being M124W, L128F, L128C, M124G, T121D, M124C, S127Y with an enrichment ratio compared to the DMSO treated population of 1/3253, 1/2493, 1/1523, 1/1288, 1/413, 1/226, 1/74 respectively. Our results agreed with our previously published electrophysiological studies (3) in which L122D, L122E and L122K were inhibited with an IC_{50}>10 microM (>1000-fold shifted; n = at least 3) compared to wild type (10 nM; 9–11 (95% confidence), n =6). The newly identified sensitive mutants M124W and L128F were inhibited by PKTHPP with an IC_{50} of 1 nM (0.9 -1.9) and 1 nM (0.5-2.1) (n = 2 and 3, each), respectively, in confirmatory electrophysiology studies. CONCLUSION: Our novel high throughput selection strategy is, as hypothesized, an effective and promising assay for studying TASK-3 pharmacology. Multiple mutations in the TASK-3 pore modify its functional interaction with PKTHPP.

Funding: Massachusetts General Hospital Department of Anesthesia, Critical Care, and Pain Medicine; NIH/HL117871; and Kantonsspital Aarau, Aarau, Switzerland (1410.000.062).

References: (1) Cotten JF. Anesth Analg 2013;116(4):10.1213. (2) Bagriantsev SN, et al. ACS Chem Biol 2013;(8)1841–1851. (2) Hietpas R, et al. Nat Protoc 2012;7(7):1382–96. (3) Chokshi RH, et al. Mol Pharm 2015;88:926–934.