Research Article

Selective Inhibition of Intracellular Kv1.3 Potassium Channels by Lentivirus-Mediated Expression of Agitoxin

Jay Yang¹*, Takeshi Suzuki¹², Maya Mikami³

¹Department of Anesthesiology, University of Wisconsin, SMPH Madison, WI, USA
²Department of Anesthesiology, Tokai University School of Medicine, Kanagawa, Japan
³Columbia University College of Physicians & Surgeons, New York, NY, USA
Supplemental Figure 1: Oligonucleotides used for creating the AgTX, scramble-AgTX, and Kv1.3 inserts. Two single stranded DNA oligonucleotides encoding the AgTX or scramble-AgTX representing the top and bottom strands were commercially synthesized (Integrated DNA Technology, Coralville, IA, U.S.A.), annealed, and kinased to create the phosphorylated double stranded inserts. The annealed inserts were designed to contain the sticky ends (underlined) for easy subcloning into the Nhe I and Not I restriction enzyme sites present in the multiple cloning site of the pLL 3.7 lentiviral vector.
Supp. Figure 2

Supplemental Figure 2: Induction of apoptosis in Jurkat cells by the sepis-mimetic cytokine stimulation.

A. TUNEL staining of the cells after 3 hrs incubation with TNFα (100 ng/ml) + cycloheximide (CHX) (500 ng/ml). Control cells (DMSO) were incubated in the same amount of DMSO (0.1%). Approximately 35% of cells were TUNEL (middle) positive and these cells showed condensed nuclei (bottom). The left panel is a phase contrast view of the same field. B. A 2% agarose gel image demonstrating DNA fragmentation by the same incubation with TNFα + CHX. C. Concentration-dependent induction of caspase 3-like activity (mean ± S.E.M.) by the same TNFα + CHX (left) or the Fas ligand (Fas-L) stimuli (right). The lines are concentration-response curve fit to the data with EC50 = 176.59± 28.32 ng/ml, Hill coefficient = 1.06± 0.14 (for TNFα) and EC50 = 0.8± 0.08 ng/ml, Hill coefficient = 1.02± 0.12 (for Fas-L). Note the complete inhibition of caspase activities by 1 µM zVAD.
Supplemental Figure 3: Transcriptional activation and release of the IL-2 and TNFα are preserved in Jurkat-AgTX cells. A. The denoted Jurkat cell lines were activated by Con A and mRNA harvested 24 hours later. The relative amounts of IL-2, TNFα, and β-actin mRNA were assayed by qRT-PCR. The amplification plots (left) were from Jurkat-EGFP (green), -AgTX (red), -scramble AgTX (purple) or -Bcl-2 (blue) mRNA templates at before (circle) and after (triangle) Con A treatment. The table (right) shows the normalized (ΔΔCt ) mean values from a triplicate measurement and the experiment was repeated 3 times with similar results. A unit positive shift in ΔΔCt value (untreated - treated) indicates a theoretical maximum of 2-fold increase in the mRNA with treatment. B. RT-PCR products for IL-2 and TNF-α amplifications confirming the correct product size and the relative amounts. The amplification cycles were stopped near half-maximum to emphasize the difference in the product amounts but the same cycle number was used for the control and experimental amplification pairs for each cell lines. C. Proinflammatory cytokines released by activated Jurkat cell lines. The cell culture medium 24 hours after stimulation with the phorbol ester + ionomycin (PMA+IoM) or concanavalin A (Con A) were assayed for IL-2 and TNFα by ELISA. The pre-stimulation levels of the cytokines for all cell lines were below detection threshold (< 2.3...
for IL-2 and <1.0 for TNFα). The numbers are mean ± S.E.M. from 6 measurements from 2 different experiments. * denotes measurements below the lower limit of detection.

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