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Initial Characterization of the Indole-3-Carboxamide Bic-154 as a Fast Onset and Reversible ORAI Channel Blocker

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account for these changes in both conductance and gating. Such studies not only expand our knowledge of the principal mechanisms underlying SF gating of K+ channels but may also help aid our understanding of how some K+ channel agonists exert their effects on the selectivity filter.

1232-Pos

Initial Characterization of the Indole-3-Carboxamide Bic-154 as a Fast Onset and Reversible ORAI Channel Blocker

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Calcium ion elevations are required for human T-lymphocyte proliferation in response to antigen recognition by a T-cell receptor. Calcium influx through the plasma membrane is necessary for efficient T-cell proliferation and effector function. The calcium channels responsible for Ca^{2+} influx in lymphocytes have been identified and Orai interacting with ER Ca^{2+} sensor STIM were shown to be crucial for persistent calcium mobilization. Loss-of-function mutations in Orai1 or STIM1 result in severe combined immunodeficiency (SCID) with muscle hypotonia. Suppression of calcium influx through Orai/STIM channels gives rise to various lymphoproliferative defects. Thus, deletion of Orai or STIM in mice results in lymphoproliferative disorders and hair loss. Gain-of-function mutations in Orai or STIM resulting in constitutively activated channels cause tubular aggregate myopathy (TAM). Despite the importance of Orai/STIM pathway in multiple signaling pathways and diseases, small molecule high affinity specific inhibitors of these channels are lacking. In this work we examined a class of indole-3-carboxamides for their potential to inhibit store-operated Ca^{2+} entry. They were tested *in vitro* for their ability to inhibit Orai1 mediated Ca^{2+} entry and currents. At 10 μM and higher the less lipophilic BIC-154 was the most potent blocker in the group. We used a constitutively active Orai1 variant implicated in TAM, which does not require STIM1 for function. BIC-154 rapidly and reversibly inhibited Orai1 currents. BTP2 (YM-58483) and 2-APB also inhibited Orai1 mutant channels in the same cells. TRPM7, a Ca^{2+}/Mg^{2+} permeable channel highly expressed in lymphocytes was insensitive to these compounds tested at 100 μM. Future investigations will test their effectiveness in T-cell blastogenesis and proliferation assays. We anticipate that Orai1-specific compounds will be useful for proof of concept studies of the contribution of this channel to human disease.

1233-Pos

Human CFTR Channel Function is Regulated by Cholesterol

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Cholesterol, a major membrane lipid component, has been known to modulate the function of multiple ion channels by specific lipid-protein interactions, by physical property changes of the membrane, or by modification of regulatory proteins associated with the channels in signaling complexes. Previous studies show that epithelial cells expressing the most common mutant version of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR), F508del-CFTR, exhibit increased cholesterol content at the plasma membrane compared to wild type control cells; however, neutrophils derived from CF patients show reduced cholesterol levels in the plasma membrane. But whether cholesterol directly modulates CFTR channel function remains unknown. To answer this question, we combine heterologous expression in oocytes with more physiologically relevant Ussing chamber recordings utilizing polarized Fischer Rat Thyroid (FRT) cells to determine the effects of changing plasma membrane cholesterol levels on CFTR channel function. Here, we report that cholesterol depletion with methyl-β-cyclodextrin (MβCD) or cholesterol oxidase (CO) has no macroscopic effect on the magnitude of CFTR-mediated whole-oocyte currents. However, depletion of cholesterol increased the effect of VX-770, a clinically-used potentiator of CFTR, when channels were activated at high PKA concentrations, but did not change the effect of VX-770 when channels were activated at low PKA concentrations. This change in efficacy of VX-770-mediated potentiation likely reflects the apparent shift in the sensitivity of WT-CFTR to PKA after depletion of membrane cholesterol. In FRT cells, P67L-CFTR also exhibited changes in phosphorylation-dependent activation after depletion of plasma membrane cholesterol. Sensitivity of WT-CFTR to block by GlyH-101 was significantly stronger after plasma membrane cholesterol depletion. These results demonstrate that changes in the cholesterol level of the plasma membrane significantly modulate multiple CFTR channel functions. (Support: CF Fdn. MCCART17G0, NIH T32 GM008602, NIH F31 HL143863-01).

1234-Pos

Activation of Potassium Channel as a New Strategy to Boost Antitumour Immune Response

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There have been substantial advances in harnessing the immune system to fight cancers using immunotherapies and engineered T cells. However, tumor-mediated immunosuppression represents a major obstacle to these approaches. Dying necrotic cells in the tumour microenvironment release substantial amount of intracellular potassium (K^+), raising extracellular K^+ ($[K^+]_e$) to 25-60 mM. Tumor-infiltrating lymphocytes (TIL) bathed in this $[K^+]_e$-rich fluid are suppressed by an ''ionic-checkpoint'' and fail to mount an efficient antitumor response. Here, we demonstrate that T cells exposed to $[K^+]_e$-rich media dose-dependently accumulate intracellular K^+. Presence of high amount of $[K^+]_e$ (50 mM) resulted in significant suppression of T cell functions, including proliferation, cytokine secretion, downstream signal transduction (Akt and mTOR pathway) and tumor killing. To test if increased K^+ efflux through K^+ channels would protect TILs from the suppressive effects of high $[K^+]_e$, we patch-clamped TILs isolated from patients with metastatic colorectal cancer that were expanded *ex vivo*. We found them to express significant numbers of calcium-activated $K_{Ca}3.1$ K^+ channels. Activators of $K_{Ca}3.1$ significantly enhanced $K_{Ca}3.1$ channel activity in both the cloned $K_{Ca}3.1$ and primary T cells, rescued T cells from high $[K^+]_e$-induced suppression in cytokine and tumor-killing. This study suggests that pharmacological activators of $K_{Ca}3.1$ enable T cells to overcome ionic-checkpoint-mediated immune suppression and mount effective antitumor activity.

1235-Pos

Novel Inhibitors of the Calcium-Activated K^+ Channel $K_{Ca}3.1$ to Treat Non-Alcoholic Fatty Liver Disease and Liver Fibrosis

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Liver fibrosis resulting from non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver-related morbidity and mortality. Thecalcium-activated$K_{Ca}3.1$ potassium channel is considered a therapeutic target for this disease because of its role in myofibroblasts and T cells, which are key drivers of fibrosis. Here, we describe the development of novel $K_{Ca}3.1$ inhibitors. Our starting template nifedipine, a dihydropyridine, blocks $K_{Ca}3.1$ with an IC_{50} = 1.8 μM. Nifedipine analogues were screened for blocking of $K_{Ca}3.1$ using automated planar electrophysiology. Structure-activity-relationship analysis led to the identification of novel analogues with IC_{50} in the low nanomolar range. Mutagenesis studies showed that Nifedipine bound to the fenestration region of $K_{Ca}3.1$, while the new analogues bound in the channel pore. Molecular modeling and simulations were used to characterize the binding of these compounds. In parallel, we tested $K_{Ca}3.1$ inhibitor TRAM-34 in mice reconstituted with a functional human immune system and fed an obesity-inducing diet. TRAM-34 prevented the progression of NALFD to liver fibrosis, reduced T cell infiltration of the liver and reduced circulating levels of IFN-γ and IL-17. These studies indicate that $K_{Ca}3.1$ blockers may have use in the treatment of diet-induced liver fibrosis.

1236-Pos

Structural Modeling of Drug Interactions with hERG Channel in Open and Closed States

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