In lymphocytes Ca^{2+} signals are essential for diverse cellular functions. After antigen binds to the T cell receptor a series of reactions are initiated that generate IP_3 and culminate in an increase in cytosolic Ca^{2+}. Mechanisms that remove Ca^{2+} also exert an important influence on the net Ca^{2+} level. The SERCA pump resequesters Ca^{2+} into the ER and the PMCA transports Ca^{2+} to the extracellular side. Two key features of the PMCA are its stimulation by Ca^{2+}-calmodulin and by PKA-dependent phosphorylation (Bers, 2001) but in most of the mathematical models this pump is represented with a simple Michaelis-Menten formulation due to its small contribution to the overall Ca^{2+} fluxes. This is not the case in T cells, Bautista et al (2002) showed that this pump is the primary means of Ca^{2+} extrusion in T cells and its activity is modulated by Ca^{2+} enabling the cell to adapt to higher Ca^{2+} values during T cell activation. Additionally once $[Ca^{2+}]_i$ returned to baseline levels the PMCA recovered slowly with a time constant of ~ 4 min providing a "memory" of previous $[Ca^{2+}]_i$. We created a first order kinetics model of the PMCA that mimics its activation and recovery as function of $[Ca^{2+}]_i$ (Vmax increased from 30 to 60 nM s^{-1}, Km decreased from 500 to 400 nM when fully activated) and incorporated into a mathematical description of Ca^{2+} signaling in T cells (LymphoLAB). Our results indicate that modulation of the PMCA activity improves the stability of Ca^{2+} signaling by adjusting the pump rate to Ca^{2+} influx even at high $[Ca^{2+}]_i$ levels (preventing a harmful Ca^{2+} overload). Moreover the delay in modulation permits small Ca^{2+} fluxes to increase transiently enhancing Ca^{2+} signaling dynamics.

1427-Pos Board B337

Potassium Channel Regulation of Thromboxane A2 Receptor Function

Shahab M. Danesh, Min Li, Enrico Stefani, Ligia Toro.

G-protein coupled receptors (GPCRs) and the large-conductance, voltage- and Ca^{2+} -activated K^+ channel (MaxiK), are commonly expressed at the plasma membrane of cells. GPCR activation results in activation or inhibition of MaxiK channel activity depending on the type of GPCR. For example, activation of Thromboxane A2 receptor leads to MaxiK channel inhibition. We now investigate whether MaxiK channel alpha subunit (Slo1) can talk back and regulate Thromboxane A2 receptor (TP) function. To this end, we analyzed two downstream processes of activated TP: activation of the monomeric G-protein Rho and stimulation of intracellular calcium release. Expression of *Slo1* in HEK293T cells increased Rho mediated transcriptional activation of a Rho specific variant of the Serum Response Factor promoter (SRE.L) by TP agonist, U46619 by 53% ($P< 0.05$). Accordingly, co-expression of the *Clostridium botulinum* C3 transferase, a Rho inhibitor, completely attenuated U46619 induced stimulation of SRE.L transcription. Expression of the regulator of G-protein signaling domain domain of p115 rhoGEF (p115 rgRGS), a blocker of TP function, also resulted in 85% ($P<0.05$) reduction in U46619 induced stimulation of SRE.L transcription. Additionally, *Slo1* expression increased TP mediated intracellular calcium release by 65% ($P<0.05$) and all intracellular calcium release was efficiently blocked by addition of TP antagonist SQ 29,548. An increase in Rho and Ca^{2+} pathways may lead to increased contraction in the vasculature where thromboxane A receptor and MaxiK channels are expressed. Moreover, the findings demonstrate a new paradigm in ion channel-GPCR signaling. Supported by NIH.

1428-Pos Board B338

Angiotensin II Type 1 receptor Associates with Maxik Channel Alpha Subunit and Inhibits its Activity

Zhu Zhang, Min Li, Enrico Stefani, Ligia Toro.

Angiotensin II (Ang II) has a large impact in cardiovascular physiology and serves as the most potent pharmacological target in the treatment of hypertension. Most of the known physiological effects of Ang II are mediated by Angiotensin II type 1 receptor (AT1 receptor). AT1 receptor and another transmembrane protein, the large conductance calcium-activated potassium (MaxiK, BK) channel, both abundantly expressed in vascular smooth muscle cells, are believed to play significant roles in regulating vascular tone. Our present studies show that co-expression of AT1 receptor with MaxiK channel results in inhibition of channel activity. AT1 receptor expression produces a 13-mV or 32-mV rightward shift of voltage necessary to half activate the channel ($V_{1/2}$). AT1 receptor was also found in co-immunoprecipitates (co-IP) with MaxiK α subunit (MaxiKα, Slo1) in HEK293T cells expressing both proteins. Consistent with co-IP results, a substantial co-localization of AT1 receptor and MaxiKα at the plasma membrane was observed using live labeling and confocal microscopy. Furthermore, the association of AT1 receptor with MaxiKα was dramatically increased in response to Ang II stimulation. These results strongly suggest a novel protein-protein interaction-based regulation of MaxiK channels by AT1 receptor, which will advance our understanding of GPCR-ion channel interaction network on the fine regulation of vascular tone. Supported by NIH.

1429-Pos Board B339

Nucleoside Diphosphate Kinase B Knockout Mice Have Impaired Activation of the K+ channel KCa3.1 Resulting in Defective T Cell Activation

Shekhar Srivastava, Lie Di, Olga Zhdanova, Yi Sun, Zhai Li, Edward Y. Skolnik.

Nucleoside Diphosphate kinases (NDPK) are encoded by the *Nme* (non-metastatic cell) gene family. While they comprise a family of 10 genes, NDPK-A and B are ubiquitously expressed and account for most of the NDPK- activity. We previously showed that NDPK-B activates the K^+ channel KCa3.1 via histidine phosphorylation of the carboxyterminus of KCa3.1, which is required for T cell receptor (TCR) stimulated Ca^{2+} flux and proliferation of activated naive human CD4 T cells. We now report the phenotype of *NDPK-B*$^{-/-}$ mice. *NDPK-B*$^{-/-}$ mice are phenotypically normal at birth with a normal life span. Although T and B cell development is normal in *NDPK-B*$^{-/-}$ mice, KCa3.1 channel activity and cytokine production is markedly defective in T helper 1 (Th1) and Th2 cells, while Th17 function is normal. These findings phenocopy studies in the same cells isolated from *KCa3.1*$^{-/-}$ mice and thereby supports genetically that NDPK-B functions upstream of KCa3.1. NDPK-A and B have been linked to an astonishing array of disparate cellular and biochemical functions, few of which have been confirmed in vivo in physiological relevant systems. *NDPK-B*$^{-/-}$ mice will be an essential tool to definitively address the biological functions of NDPK-B. Our finding that NDPK-B is required for activation of Th1 and Th2 CD4 T cells, together with the normal overall phenotype of *NDPK-B*$^{-/-}$ mice, suggests that specific pharmacological inhibitors of NDPK-B may provide new opportunities to treat Th1 and Th2 mediated autoimmune diseases.

1430-Pos Board B340

Distinct Regulation of Slo1 Human Gene by Estrogen and Src

Shahab M. Danesh, Pallob Kundu, Enrico Stefani, Ligia Toro.

Estrogen Receptor Alpha (ERα) is a transcription factor for several genes via genomic or non-genomic (signaling) mechanisms. We previously showed that activated ERα induces transcription of the murine large-conductance, voltage- and Ca^{2+} -activated K^+ channel (*mSlo1*) gene through a genomic-dependent mechanism. Here, we analyzed whether ERα transcribes *humanSlo1 (hSlo1)* gene. ERα transcription of the *hSlo1* gene takes place at a much lower estrogen (E2) concentration (EC_{50}=0.07 nM) than for *mSlo1* (EC_{50}=0.4 nM), suggesting a different mechanism of *hSlo1* activation by ERα. Accordingly, disruption of the DNA binding domain of ERα or knockdown of Sp1 protein as a potential genomic mediator of ERα effect did not alter the up-regulation of *hSlo1* in response to E2. These results rule out direct and Sp-factor -and AP-1 factor- (absent in *hSlo1* promoter)-mediated genomic action of ERα towards hSlo1 transcription. To identify the non-genomic mechanism involved in ERα transcriptional action, three candidate pathways (Rho, PI3 Kinase (PI3K), and Src tyrosine kinase) were explored utilizing DNA binding domain mutant (DBDM) variant of ERα. Inhibition of Rho function by co-expression of Clostridium toxin transferase (C3) had no effect on E2 action. However, inhibition of PI3K activity with 10 μM LY294002 resulted in a 44% decrease in E2-stimulated *hSlo1* expression ($P<0.05$). Interestingly, blockade of Src activity with 10 μM PP2 did not inhibit E2 action but caused a 2 fold increase in the basal level of *hSlo1* transcription to levels attained with E2 activation ($P<0.05$). Conversely, constitutively active cSrc (Y527F) completely abolished basal and E2 mediated activation of *hSlo1* transcription. These results indicate that PI3K plays a role in E2 mediated *hSlo1* gene expression while Src activity does not mediate E2-induced *hSlo1* transcription but serves as a repressor of *hSlo1* gene expression. Supported by NIH.

1431-Pos Board B341

Paxilline, a Closed BK Channel Blocker

Yu Zhou, Christopher J. Lingle.

The tremorogenic fungal metabolite, paxilline, is widely used as a potent and relatively specific blocker of Ca^{2+}- and voltage-activated Slo1 (or BK) K^+ channels. Little is known about the mechanism of paxilline blockade, although paxilline has been proposed to act in an allosteric manner to influence the closed-open equilibrium and block by paxilline has been shown to be Ca^{2+} -dependent, with weakened block at more elevated Ca^{2+}. Our previous study shows that block by paxilline is absolutely dependent on the presence of Glycine311 in the BK S6 helix, suggesting that block may occur within the BK