may modify the voltage activation threshold of this channel towards more physiological conditions. Flavonoid Naringenin (Nar) is present in all plant species where it plays a central role in the flavonoid biosynthetic pathway. Nar is stored in the vacuoles in glycosylated form. To confirm the presence of non-glycosylated Nar in the cytoplasm we isolated the gene encoding for Arabidopsis glycosyltransferase (AtGT) which glycosilates Nar. AtGT gene was cloned in fusion with yellow fluorescent protein (YFP) and was used for localization studies. When Naringenin was added to cytosolic bath solution, we recorded a dose-dependent reversible decrease in SV channel activity described by a half block concentration of 0.44 mM. Investigating Nar effects on the voltage dependence of the channel, we observed that the activation threshold of the SV channel is shifted towards more positive voltages and that Nar does not affect the single channel conductance. Investigating the effects of Nar at varying pH, we observed an increase in current inhibition with the decrease of the pH. When Naringin, the glycosylated form of Nar, was applied at the cytosolic or at the vacuolar side it did not modify the channel activity. We are currently investigating the role of the phospholipid composition of the membrane in this modulation.

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**2761-Pos**

**Effects of Cannabinoids on Ion Channels of Pancreatic Beta Cells**

**Charles E. Spivak**[1], Máire E. Doyle[2].
[1]NIDA/IRP, Baltimore, MD, USA, [2]Johns Hopkins Medical Institutes, Baltimore, MD, USA.

The function of the beta cell of the pancreatic islet is to sense minute changes in blood glucose levels and secrete insulin to maintain euglycemia. Though this secretion is governed chiefly by glucose, other chemical factors such as insulin itself and the incretin hormones also modulate this function. Peliminary evidence in our lab indicates that endocannabinoids are important negative regulators of insulin secretion. Beta cells are electrically excitable and undergo depolarization upon glucose stimulated insulin secretion resulting from closure of the K(ATP) channels. As a consequence, the calcium channels open, intracellular levels of calcium rise and exocytosis of the insulin secretory granules occurs. In addition to the K(ATP) channels and the calcium channels, beta cells also possess an array of ion channels that generate the complex electrical waveforms and participate to varying degrees in the regulation of the membrane potential.

Using rodent beta cells we investigated the effects of cannabinoids on some of these ion channels. Thus, 10 μM 2-arachidonylglycerol (2-AG) decreased the amplitude of the delayed rectifier current by about 40%. Simple washing did not reverse this blockade, and the addition of the cannabinoid receptor 1 (CB1) antagonist AM 251 (1 μ) also had no effect. However, the blockade was completely reversed by washing with lipid free bovine serum albumin. Moreover, the CB1 agonist, WIN-55,212-2(1 μM ), an indole derivative structurally unrelated to the lipid 2-AG, had no antagonistic effect on the delayed rectifier. Taken together, these results suggest that the block was independent of the CB1 receptor and was mediated instead via the plasma membrane. Cannabinoid blockade of both sodium and HVA calcium currents were similar to the effects on the delayed rectifier.

**2762-Pos**

**Effects of Small Molecule Kv1.3 and K(Ca)3.1 Inhibitors on $T_{EM}$-Cell Proliferation**

**Tobias Dreker**, Svetlana Hamm, Stefan Tasler.
4SC AG, Munich, Germany.

The homotetrameric forms of the voltage- or calcium-activated Kv1.3 and K(Ca)3.1 channels are the predominant potassium channels in T-lymphocytes and play an important role in membrane potential regulation of these cells. Since several years Kv1.3 has been suggested as a promising target for addressing autoimmune diseases because Kv1.3$^{high}$/K(Ca)3.1$^{low}$phenotype T-lymphocyte subsets (activated $T_{EM}$ cells) are thought to play an important role in the mediation of the pathologic effects.

We developed several small-molecule compounds with Kv1.3 or K(Ca)3.1 inhibitory properties in the nanomolar range determined by manual patch-clamp experiments. To further test the efficacy of the compounds on a cellular level we tried to suppress $T_{EM}$ subtype specific anti-CD3 induced proliferation of freshly isolated PBM, T, and $T_{EM}$ cells (fraction of purified T and $T_{EM}$ cells characterized by FACS detection of CD45RO and CCR7 antigens). Inhibition of proliferation was either essayed by exclusive application of 4SC or reference Kv1.3 inhibitors or by co-application in combination with a K(Ca)3.1 inhibitor. In all cases inhibition of Kv1.3 potassium channels alone did not or only weakly abolish cell proliferation. However, co-inhibition of Kv1.3 and K(Ca)3.1 channels widely suppressed proliferation to various degrees. Furthermore we observed that the degree of block seemed to be strongly dependent on the donor and/or the individual's immunological status. This potentially T-cell subset unspecific inhibition has recently been suggested as a novel strategy in preventing kidney allograft rejection (*Transplant. Proc.* (2009) **41**:2601-2606).

**2763-Pos**

**Effects of PKC on Closed-State Inactivation in Kv4.3 Isoforms**

Chang Xie, Vladimir E. Bondarenko, Harold C. Strauss, **Michael J. Morales**.
UB, SUNY, School of Medicine, Buffalo, NY, USA.

Kv4.3 is expressed as two isoforms, a short form and a long form, that has a 19 aa insertion downstream from S6 in the C terminus which has a putative PKC phosphorylation site at T504. To understand the role of PKC on modulation of closed-state inactivation (CSI), we expressed channels mutated in putative PKC phosphorylation sites and compared their response to PKC activation with PMA to the responses of WT Kv4.3 isoforms. PMA had similar effects on Kv4.3-S and Kv4.3-L open-state inactivation. However, PMA induced opposite effects on CSI in the two channel splice variants: the magnitude of CSI in Kv4.3-S was reduced, while there was an increase in CSI in Kv4.3-L, an effect was abolished by mutation of the long form T504 to alanine. To understand the structural basis of the reduction of CSI in Kv4.3-S, we constructed several mutants of putative PKC phosphorylation sites in the N terminus. Of these, the largest effect on PKC modulation of CSI occurred in the T53A mutant in both Kv4.3-S and Kv4.3-L; Kv4.3-S mutants lacking threonine at position 53 showed no or minimal response to PKC. These data show that isoform-specific modulation of CSI by PKC in Kv4.3 involves complex interactions of the cytoplasmic N and C termini of the channels.

**2764-Pos**

**$Ca^{2+}$-Dependent PKC Facilitates Voltage-Dependent Activation of IKs Through Phosphorylation of An Isoform Specific Site on the KCNE1 Subunit**

**Jin O-Uchi**, Elena Fujiwara, Alessandra Matavel, Coeli M. Lopes.
University of Rochester, Rochester, NY, USA.

Protein kinase C (PKC) regulates heart inotropy and chronotropy in both physiological and pathophysiological states. In human heart, at least 6 PKC isofoms are expressed, with the $Ca^{2+}$ dependent isoforms (classic PKCs, cPKCs) being the most abundant. However, little is known about the effect of cPKC on heart rhythm and cardiac ion channel regulation. The slow delayed rectifier current (IKs) is one of the main currents responsible for cardiomyocyte repolarization. In this study, we investigated the regulation of human IKs regulation by cPKC.Human IKs channel (KCNQ1 and KCNE1) and $\alpha_1$-adrenergic receptor were co-expressed in HEK293T cells. IKs was measured by conventional whole-cell and perforated patch-clamp techniques. The selective $\alpha_1$-adrenergic agonist phenylephrine (30 μM) activated IKs by both shifting the voltage dependence of activation ($V_{1/2}$) to the left, ~ −20 mV, and increasing in the maximal conductance (Gmax), ~ 175%. Pretreatment with cell-permeable cPKC inhibitory peptide selectively blocked the agonist-induced voltage shift, but not the increase in Gmax. Application of a cell-permeable cPKC activator peptide mimicked the agonist-induced leftward shift in $V_{1/2}$, and showed no increase in Gmax. A mutation in a putative PKC phosphorylation site in the auxiliary subunit, KCNE1(S102A), abolished the cPKC-mediated voltage shift. Expression of the phosphorylation-mimicking mutant, KCNE1(S102E), produced channels that had a leftward shift in $V_{1/2}$ compared to KCNE1(S102A). Our data indicate that cPKC phosphorylation of KCNE1(S102) facilitates voltage-dependent activation of IKs . In addition, we showed that a mutation associated with Long QT type1 at the S4-S5 linker of KCNQ1 and associated with high cardiac risk, also abolished cPKC activation of this channel. Our results suggest that KCNE1(S102) phosphorylation is transduced through the KCNQ1(S4-S5) linker to modulate channel voltage sensing and thereby facilitate channel opening.

**2765-Pos**

**Ancillary Subunits Regulate PKC Mediated Effects on Closed-State Inactivation of Kv4.3**

**Chang Xie**, Michael J. Morales.
University at Buffalo-SUNY, Buffalo, NY, USA.

Kv4 channels are expressed with a variety of ancillary subunits in vivo. The most prominent of these proteins are the KChIPs, a family of cytoplasmic proteins that modulate channel gating and act as chaperones. We have previously shown that heterologously expressed Kv4.3 is regulated by PKC. After induction of PKC by PMA, current expression level is reduced. PKC also influences closed-state inactivation (CSI) in an isoform dependent manner; CSI is decreased upon PKC induction in Kv4.3-S, and increased in PKC-L. To understand the role of PKC on modulation of Kv4-based currents, we expressed channels in the presence of three KChIP2 isoforms and compared their responses to PKC. Two KChIP2 isoforms, KChIP2a and 2b, negated PKC influence on channel gating, kinetics, and expression levels in both Kv4.3-S and

Kv4.3-L. In contrast, 70-amino acid KChIP2d was permissive for PKC modulation with respect to both CSI and current expression, while allowing the fast recovery from open-state inactivation characteristic of other KChIP2 isoforms. Additionally, the KChIP2d effects on CSI in Kv4.3-L were dependent on the presence of a putative PKC phosphorylation site in the C terminus. These data suggest a different physiological role for KChIP2d than the other KChIP2 isoforms, and suggest that the longer forms of KChIP2 interact with the regions of Kv4.3 affected by PKC, while KChIP2d interacts with the channel in a manner that allows PKC modulation while still accelerating recovery.

**2766-Pos**

**Regulation of NALCN Sodium Leak Channel by UNC79 and UNC80**

**Dejian Ren**[1], Boxun Xu[1], Qi Zhang[1], Haikun Wang[1], Manabu Nakayama[2].
[1]Univ Pennsylvania, Philadelphia, PA, USA, [2]Kazusa DNA Research Institute, Kisarazu, Japan.

In addition to the selective ion channels such as voltage-gated $K^+$ ($K_V$), $Na^+$ ($Na_V$) and $Ca^{2+}$ ($Ca_V$) channels, neurons also express a voltage-independent, non-selective cation channel NALCN. NALCN contributes to the TTX- and $Cs^+$ -resistant $Na^+$ leak conductance in neurons and is activated by neuropetides substance P and neurotensin via a G-protein independent pathway that requires the Src family of tyrosine kinases. The pore-forming subunit of NALCN resembles that of the 24 transmembrane spanning (24TM) $Ca_V$s and $Na_V$s, but lacks some of the charged residues in the S4s and has a unique sequence signature in the selectivity filter region. Unlike those of other ion channel families, the subunit composition of NALCN is not known. Recent studies in *Drosophila melanogaster* and *C. elegans* suggest genetic interaction between *Nalcn* and two novel genes *Unc79* and *Unc80.* We have now analyzed the mammalian homologs of UNC79 and UNC80 from mouse brain. UNC79 and UNC80 encodes large proteins (~3,000 amino acids), have no obvious domains with defined function, and are well conserved from humans "down" to the placozoan *Trichoplax adhaerens*. UNC79 and UNC80 form a complex with NALCN in the brain, where UNC79 indirectly associates with NALCN through its interaction with UNC80. While UNC79 and UNC80 are not required for the basal $Na^+$ leak current through NALCN, UNC80, but not UNC79, is essential for the channel's regulation by GPCRs. These data suggest that UNC79 and UNC80 are likely "auxiliary subunits" of the NALCN channel complex.

**2767-Pos**

**One Sumo is Sufficient to Silence the Dimeric Background Potassium Channel K2P1**

**Leigh D. Plant**, Leandro Zúñiga, Sonia Olikara, Steve A.N. Goldstein.
Univerisity of Chicago, Chicago, IL, USA.

SUMO, a 101 residue peptide well-known to regulate nucleocytoplasmic trafficking and function of transcription factors, was recently shown to reversibly regulate the activity of K2P1 channels in cell surface membranes (Rajan et al, Cell **121**; 2005). Thus, K2P1 channels are silenced by interaction of SUMO with lysine at position 274 (K274) and activated by SUMO-specific protease (SENP1). As such, channels with K274 altered to glutamine (K274Q) are constitutively active and insensitive to SUMO and SENP1. Here we report that, like wild-type K2P1 channels (WT), channels formed by two subunits linked in tandem (WT-WT) are silent at baseline and activated by exposure to SENP1 when studied in CHO cells by patch-clamp recording. Suggesting that channel silencing requires only one SUMO, channels bearing one wild-type subunit (WT-K274Q and K274Q-WT) behave like WT. To test this hypothesis, GFP-labeled subunits were studied using total internal reflection microscopy and stepwise decreases in fluorescence due to single-particle photobleaching (SPPB) to count the number of fluorophores per channel. Validating the method, two bleaching steps are recorded with GFP fused to WT or K274Q subunits because K2P channels are dimeric (Lopes et al., 2001. JBC **276**:24449-52; Kollewe et al., 2009. JGP **34:**53-68) and four steps seen with GFP on Kv2.1 subunits that form tetrameric channels. Next, GFP-SUMO was observed in discrete plasma membrane particles when expressed with WT but not K274Q subunits. Finally, SPPB was used to identify two GFP-SUMO with each WT or WT-WT channel but only one with WT-K274Q or K274Q-WT channels. The data show K2P1 channels to assemble with two SUMO subunits but a single SUMO to be sufficient for silencing.

**2768-Pos**

**Identification of $K_v$ Subunits Underlying the Delayed Rectifier $K^+$ Current ($I_K$) in Small Cultured DRG Neurons**

**Elke Bocksteins**, Gerda Van Vijver, Tine Bruyns, Pierre-Paul Van Bogaert, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Voltage-gated potassium (Kv) channels serve a wide range of functions in both excitable and non-excitable cells. In neurons these include the regulation of the resting membrane potential and control of the shape, duration and frequency of action potentials. The large number of Kv subunits presents a challenge to determine the molecular composition of the native currents. We attempted to identify the Kv subunits underlying the delayed rectifier current ($I_K$) in cultured small mouse dorsal root ganglia (DRG) neurons. Using extracellular Stromatoxin (ScTX) and intracellular Kv2.1 antibodies we recently reported that approximately 60% of the $I_K$ current in these DRG neurons is carried by both homotetrameric Kv2.1 and heterotetrameric Kv2.1/silent Kv channel complexes. The 40% of $I_K$ remaining after ScTx (100 nM) pretreatment, was virtually abolished with 1 mM extracellular TEA ($n = 6$) indicating that this part of the $I_K$ current could be represented by the Kv subunits Kv1.1, Kv1.6, Kv3.1, Kv3.2 and/or Kv3.3, and possibly a fraction of KCNQ2 and KCNQ2/3 channels, which underlie the M-current in small DRG neurons. Using channel specific toxins we determined the contribution of each channel to the remaining 40% of $I_K$. Furthermore, we detected the presence of Kv3.1, Kv3.2 and Kv3.3 mRNA using RT-PCR in freshly isolated DRG. These observations support a substantial role of at least the Kv3.x subunits in small DRG neurons which are visceral and somatic sensory neurons that conduct information about temperature, pressure and touch.

## Ion Channels, Other I

**2769-Pos**

**Tubulin-Binding Drugs Thiocolchicoside and Taxol Permeabilize Lipid Bilayer Membranes by Forming Ion Channels**

**Md. Ashrafuzzaman**[1], M. Duszyk[2], J. Tuszynski[1,3].
[1]Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada, [2]Department of Physiology, University of Alberta, Edmonton, AB, Canada, [3]Department of Physics, University of Alberta, Edmonton, AB, Canada.

The purpose of this study was to examine the possibility of ion channel formation by two tubulin-binding drugs: thiocolchicoside (TCC) and taxol (TXL). Both these compounds, in addition to interaction with tubulin, are known to have other cellular targets. For example, TCC has been shown to interact with $GABA_A$ and strychnine-sensitive glycine receptors, while TXL interferes with the normal breakdown of microtubules during cell division, inducing mitotic block and apoptosis and found significant chemotherapeutic applications in breast, ovarian and lung cancer. In order to better understand the diverse mechanisms of TCC and TXL actions, we examined their effects on phospholipid bilayer membranes formed by applying the lipid cocktail of POPE: POPS: POPC (5:3:2, v/v/v). Our electrophysiological recordings across membranes constructed in NaCl aqueous phases consisting of ~ 50 μχg/ml of TCC/TXL suggest that both of these drug molecules induce stable (possibly toroidal type) ion channels in membranes. The discrete conductance events appear with conductances (~0.01-0.1 pA/mV) and lifetimes (~5-30 ms) falling in the average orders observed in gramicidin A and alamethicin channels. The channel formation probability increases linearly with TCC/TXL concentration and transmembrane potential and is not affected by pH (5.7 - 8.4). Results suggest that TCC/TXL can partition through membranes and perhaps act at cellular levels. This novel finding may help to understand the biophysical properties of these two important drug molecules and similar ones which will hopefully assist in developing novel drugs to treat health problems related to muscular spasms, rheumatologic, orthopedic, traumatologic disorders in addition to a broad spectrum of cancers.

**2770-Pos**

**Towards Simultaneous Single Channel Current and Fluorescence Recordings in Planar Lipid Bilayer**

**Hugo McGuire**, Rikard Blunck.
Département de physique et Groupe d'Étude des Protéines Membranaires, Université de Montréal, Montréal, QC, Canada.

The ion conducting pore of $K^+$ channels has two gates – one at the selectivity filter and another at the helical bundle crossing. Both gates have to open for ions to pass through the pore. Opening of the helical bundle crossing, which is linked to the voltage sensor movement in voltage-dependent channels, is thought to occur in one cooperative step of all four subunits. On the other hand, subconductance levels, which are states of lower conductance than the normal conducting state, are suggested to be caused by a partial (i.e. not all four subunits) opening of the channel. It remains unknown whether full opening is necessary or whether partial opening is sufficient to get ion conduction. Here, we present our development towards studying the correlation between the subunits' opening and the associated current of the channel. We are investigating purified KcsA channels fluorescently labeled at the helical bundle crossing. The channels are reconstituted at low concentration in lipid vesicles and inserted into horizontal planar lipid bilayer until single channel current is observed. In the horizontal bilayer configuration, we have optical access and electrical control simultaneously. Using fluorescence spectroscopy techniques, we