electrophysiology in cultured rat striatal neurons and transfected HEK 293 cells. We determined that 79 ± 3% of cultured cells endogenously expressed β3, and 94% of neurons gave tonic-level GABA-evoked currents blocked by phasic concentrations of dopamine (0.1-10 μM). Inhibition was recapitulated in HEK 293 cells transfected with zβ3, zβ3/zα2 or zβ2/zα2 subunits, but we instead observed potentiation in zβ1/z2 and zβ3/z2. Surprisingly, dopamine (1 mM) evoked rapid currents in zβ2/z2, zβ3/z2 and zβ3/z2 in the absence of GABA. In zβ3/z2 (H267A) phy2 (z2/z2) receptors insensitive to trace Zn2+ inhibition, we found that other biogenic amines evoked comparatively smaller currents than DA. When the ratio of z1 was increased or we mutated a critical Zj2 tyrosine residue (Y62L), relative dopamine responses decreased. Furthermore, dopamine activity was retained but GABA activity was drastically reduced in Zβ3/z2 receptors, while Zj3 or γ2 alone did not elicit currents. Finally, dopamine currents were picROTOXIN- but not bicuculline- or gabazine-sensitive. Taken together, dopamine at phasic levels is a GABA allosteric modulator that may inhibit tonic GABA in prototypical z-containing GABA receptors. However, at βγ-containing receptors that do not need z subunits, dopamine acts as a positive allosteric modulator.

**2180-Pos Board B317**

**MmT1X and MmT2X from Coral Snake Venom Potently Modulate GABA(A) Receptor Activity**

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GABA(A) receptors shaped synaptic transmission by modulating Cl- conductance across the cell membrane. Remarkably, animal toxins that target GABA(A) receptors have not yet been identified. Here, we report the discovery of MmT1X and MmT2X, two toxins present in Costa Rican coral snake venom that bind to GABA(A) receptors at nanomolar concentrations. Studies with recombinant and synthetic toxin variants on hippocampal neurons and cells expressing common receptor compositions suggest that MmT1X and MmT2X potentiate GABA(A) receptor opening and accelerate desensitization when an agonist is present. In concert, hippocampal neuron excitability measurements reveal a toxin-induced transitory network inhibition followed by an increase in spontaneous activity. Moreover, toxin injections into mouse brain result in reduced basal activity between intense seizures. Altogether, we characterized the first animal toxins that potently modulate GABA(A) receptor function, thereby establishing a novel class of tools to study this receptor family.

**2181-Pos Board B318**

**Monitoring Motions in GABA-A Receptor Intersubunit Transmembrane Cavities**

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GABA-A receptors ((GABAr, Rs) are the target for many therapeutic drugs including benzodiazepines (BZDs), barbiturates, anesthetics and neurosteroids. These drugs bind to distinct sites and potentiate GABA-induced currents. They are often used in combination but little is known about mechanism(s) underlying their interactions. Recent crystal structures of GLIC and the glutamate-activated chloride channel suggest agonist-mediated channel opening is associated with expansion of transmembrane interfaces between M3 and M1 helices of adjacent subunits. Here, we examined if binding of different allosteric drugs and combinations of these drugs to z1b2γ1, GABAr, Rs induces similar motions. We also tested whether potentiation of GABA-mediated current by pairs of drugs binding to distinct sites are additive or super-additive.

We individually inserted a cysteine in the M1 helices of each GABAr, Rs subunit at γ1L227, β2L223 and γ2L238 to monitor motions at different intersubunit interfaces. We expressed wild-type and mutant GABAr, Rs in Xenopus oocytes. We measured rates of modification of the substituted cysteines in the absence and presence of different combinations of allosteric modulators that bind to different sites: the intravesicular anesthetics pentobarbital (PB) and etomidate, the neurosteroid THDCC and the benzodiazepine flurazepam. When applied alone, flurazepam and THDCC induced distinct motions in the intersubunit interfaces, flurazepam increased rate of modification of only γ1L238 whereas THDCC only increased the rate of βL223. When flurazepam and THDCC were co-applied, no change in rate of modification of γ1L238 was observed indicating that the presence of THDCC inhibited the ability of flurazepam to elicit structural rearrangements near γ1L238. The data suggest structural mechanisms underlying the effects of combining drugs are different than mechanisms underlying their actions alone.

**Ion Channel Regulatory Mechanisms II**

**2182-Pos Board B319**

**Liberation of Pser68-Plm Inhibition of NCX1 by an Optimized Anchoring Disruptor Peptide**

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The cardiac sodium-chloride exchanger (NCX1) is an important regulator of intracellular Ca2⁺ ([(Ca2⁺)]i) and a potential therapeutic target in heart failure. Among potential interacting partners regulating NCX1 activity is the transmembrane protein phospholemman (PLM); a substrate for protein kinase A and protein kinase C. Several reports have demonstrated that binding of phosphorylated PLM (pSer68-PLM) to NCX1 leads to NCX1 inhibition, while other studies have failed to demonstrate a functional interaction. In present study, we aimed to analyze the biological function of the pSer68-PLM-NCX1 interaction by developing anchoring disrupter peptides. We observed that PLM co-fractionated and co-immunoprecipitated with NCX1 in rat left ventricle (LV) and in co-transfected HEK293 cells. Strong binding of pSer68-PLM to one of the previously reported NCX1-QKHPD regions was demonstrated in pull-down and overlay assays. Single amino acid substitutions of native NCX1 peptide sequence 1-KHPDEIEQLANYQVLS-20 revealed that single substitutions at position 1-11 (particularly with tryptophan or tyrosine) increased binding affinity of the peptide to pSer68-PLM. Interestingly, we found that double substitution at position 4 and 6 with tyrosine (D4E6Y) in the native peptide sequence enhanced the binding affinity to pSer68-PLM 8-fold, compared to native sequence. The optimized peptide 1-KHPKVEIEQLANYQVLS-20 was further tested in a series of electrophysiological experiments using whole-cell voltage clamp technique, with voltage-ramp protocol employed to elicit NCX current. Constitutively phosphorylated (S66D) PLM was observed to inhibit NCX1 current in both forward and reverse mode. Inclusion of the disrupter peptide in the patch pipette reversed S66D-PLM inhibition of NCX1, while NCX current was not altered by a scrambled peptide control. Taken together these data strongly suggest that PLM interacts directly with NCX1, and that PLM is phosphorylated at serine 68, the interaction inhibits NCX1 activity.

**2183-Pos Board B320**

**The Talk-1 C-Terminus is a Charge-Sensitive Module Regulating Channel Activity**

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Human beta-cell insulin secretion is dependent on Ca2⁺ entry through voltage-dependent Ca2⁺ channels (VDCCs). K⁺ channels modulate the beta-cell membrane potential, which influences VDCC activity. The most abundant K⁺ channel of the human islet is the two-pore domain K⁺ (K2P) channel TALK-1; however, its function is unknown. We characterized TALK-1 currents in primary mouse beta-cells and find that K2P currents are significantly reduced after genetic ablation of TALK-1. To identify mechanisms that regulate TALK-1 channel activity, we used a Ti⁺-sensitive fluorescent screen to assess if TALK-1 is regulated by kinases. Cells expressing TALK-1 channels were monitored for changes in Ti⁺ flux in response to co-expression with a constitutively active kinase (examining a total of 192 kinases), revealing 9 kinases that modify TALK-1 activity. Among potential interacting partners regulating NCX1 activity is the transmembrane protein phospholemman (PLM); a substrate for protein kinase A and protein kinase C. Several reports have demonstrated that binding of phosphorylated PLM (pSer68-PLM) to NCX1 leads to NCX1 inhibition, while other studies have failed to demonstrate a functional interaction. In present study, we aimed to analyze the biological function of the pSer68-PLM-NCX1 interaction by developing anchoring disrupter peptides. We observed that PLM co-fractionated and co-immunoprecipitated with NCX1 in rat left ventricle (LV) and in co-transfected HEK293 cells. Strong binding of pSer68-PLM to one of the previously reported NCX1-QKHPD regions was demonstrated in pull-down and overlay assays. Single amino acid substitutions of native NCX1 peptide sequence 1-KHPDEIEQLANYQVLS-20 revealed that single substitutions at position 1-11 (particularly with tryptophan or tyrosine) increased binding affinity of the peptide to pSer68-PLM. Interestingly, we found that double substitution at position 4 and 6 with tyrosine (D4E6Y) in the native peptide sequence enhanced the binding affinity to pSer68-PLM 8-fold, compared to native sequence. The optimized peptide 1-KHPKVEIEQLANYQVLS-20 was further tested in a series of electrophysiological experiments using whole-cell voltage clamp technique, with voltage-ramp protocol employed to elicit NCX current. Constitutively phosphorylated (S66D) PLM was observed to inhibit NCX1 current in both forward and reverse mode. Inclusion of the disrupter peptide in the patch pipette reversed S66D-PLM inhibition of NCX1, while NCX current was not altered by a scrambled peptide control. Taken together these data strongly suggest that PLM interacts directly with NCX1, and that PLM is phosphorylated at serine 68, the interaction inhibits NCX1 activity.