cell patch-clamp technique and $Ca^{2+}$ signals and ROS production were measured with the fluorescent probes, Fluo 3-AM and CM-$H_2$DCFDA, respectively. The levels of the $\alpha_{1c}$ subunit, obtained from diazoxide preconditioned hearts, were measured in the membrane fraction of rat ventricles by Western blot. The ROS scavenger NAC was used to examine the role of ROS on the L-type $Ca^{2+}$ channel after PP in both preparations.
**Results**: Diazoxide induced PP was accompanied by a significant downregulation of the $\alpha_{1c}$ subunit in the membrane fraction and by a reversible reduction in the amplitude of $I_{Ca}$ and $Ca^{2+}$ transients. These effects were complete within 90min and were prevented by NAC. Diazoxide significantly increased ROS production in cardiomyocytes. The reduction of $I_{Ca}$ and $Ca^{2+}$ transients by PP were prevented by the mitochondrial $K_{ATP}$ channel blocker 5-HD.
**Conclusions**: Pharmacological preconditioning induced with diazoxide, leads to downregulation of the $\alpha_{1c}$ subunit of the L-type $Ca^{2+}$ channel. This reduces the influx of $Ca^{2+}$ through these channels and may contribute to attenuate the overload of $Ca^{2+}$ during reperfusion.
**Grants**: CONACYT: 60880 (J.S) and 82667 (M.C.G).

**2680-Pos**
**Voltage-Dependent Kappa Opioid Modulation of Calcium Currents Elicited by Action Potential Waveforms in Neurohypophysial Terminals**
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Release of neurotransmitter is activated by the influx of calcium. Inhibition of $Ca^{2+}$ channels results in less calcium influx into the terminal and, presumably, a reduction in transmitter release. In the nerve terminals of the neurohypophysis, voltage-gated calcium channels (VGCC) are primarily controlled by membrane voltage and their activity can be modulated, in a voltage-dependent manner, by their interaction with G-protein subunits. Endogenous opioids also affect (inhibit) these calcium channels, upon binding to μ- and κ-receptors at the terminals.
Voltage-dependent relief of G-protein inhibition of VGCC is achieved with either a depolarizing square pre-pulse or by action potential waveforms. Both protocols were tested in the presence and absence of opioid agonists targeting the μ- and κ-receptors. The κ-opioid VGCC inhibition is relieved by such pre-pulses, suggesting that this receptor is involved in a voltage-dependent membrane-delimited G-protein pathway. In contrast, μ-opioid inhibition of VGCC is not relieved by such pre-pulses, indicating a voltage-independent diffusible second-messenger signaling pathway. Furthermore, κ-opioid inhibition is also relieved during stimulation with action potential bursts with physiological characteristics. This indicates the possibility of activity-dependent modulation *in vivo*.
Differences in the facilitation of $Ca^{2+}$ channels due to specific G-protein modulation during a burst of action potentials may contribute to the fine-tuning of $Ca^{2+}$-dependent neuropeptide release in other central nervous system synapses, as well. [Supported by NIH Grant NS29470].

**2681-Pos**
**Selective Inhibition of T-Type Calcium Channels by Endogenous Lipoamino Acids**
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T-type calcium channels, i.e. Cav3.1, Cav3.2 and Cav3.3 channels, have important roles in cell excitability and calcium signalling and contribute to a wide variety of physiological functions especially in nervous system. Over the past few years, several endogenous ligands regulating Cav3 activity were identified, including bioactive lipids such as the endocannabinoid anandamide (N-arachidonoyl ethanolamine). We now provide evidence that the T-type / Cav3 calcium channels are potently and reversibly inhibited by various lipoamino acids, including N-arachidonoyl glycine (NAGly, IC50 ~ 600 nM for Cav3.2) and N-arachidonoyl 3-OH-gamma-aminobutyric acid (NAGABA-OH, IC50 ~200 nM for Cav3.2). This inhibition involves a large shift in the Cav3.2 steady-state inactivation and persists during fatty acid amide hydrolase (FAAH) inhibition as well as in cell-free outside-out patch. It appears that lipoamino acids are the most active endogenous ligand family acting on T-channels. Importantly, lipoamino acids have weak effects on high-voltage-activated (HVA) Cav1.2 and Cav2.2 calcium currents, on Nav1.7 and Nav1.8 sodium currents as well as on TRPV1 and TASK1 currents. These data indicate that lipoamino acid effects may be selective of T-type channels over HVA calcium channels, sodium channels as well as the anandamide-sensitive TRPV1 and TASK1 channels. It also suggests that these ligands can modulate multiple cell functions via T-type calcium channel regulation. In line with this, we found that lipoamino acids evoke a thermal analgesia in wild-type but not in Cav3.2 KO mice. Collectively, our data identify lipoamino acids as a new potent and selective family of endogenous T-type channel inhibitors.

**2682-Pos**
**Chronic Alcohol Consumption Blunts β-Adrenergic Responsiveness in Left Ventricular Cardiomyocytes**
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Alcoholic cardiomyopathy (ACM) develops from long-term, excessive consumption of alcohol. Initially, ACM is asymptomatic but continued alcohol abuse leads to reductions in cardiac contractility, the onset of arrhythmias, chamber dilation and congestive heart failure. This study was carried out to examine the effects of chronic alcohol on basal and β-adrenergic-stimulated properties of $Ca^{2+}$ transients during excitation-contraction (E-C) coupling. Rats were pair-fed DeCarli and Lieber control and alcohol liquid diets for 120 days prior to isolating left ventricular myocytes. Under basal conditions, there was no change in the amplitude of electrically-triggered $[Ca^{2+}]_i$ transients (Control, $296 \pm 21$nM vs. Alcoholic, $260 \pm 18$ nM) or contraction (Control, $11.9 \pm 0.6$ μm, Alcoholic,$12.7 \pm 1.2$ μm. However, a blunted inotropic response (increase over basal: Control, $90 \pm 19$% vs. Alcoholic $39 \pm 10$ %) was observed in the presence of submaximal isoproterenol stimulation. In addition, maximal isoproterenol and forskolin stimulation do not improve the inotropic response of the alcoholic myocytes, suggesting a functional impairment in the initial $Ca^{2+}$ release steps of E-C coupling. Consistent with the reduced $[Ca^{2+}]_i$ transient amplitude, the $Ca^{2+}$ current ($I_{Ca,L}$) responses to isoproterenol were also markedly reduced in cardiomyocytes from alcohol-fed animals. Surprisingly, measurement of L-type calcium channel expression by dihydropyridine (DHP) binding and real-time PCR, revealed an increased number of DHP binding sites (Control $B_{max} = 197 \pm 60$ fmol/mg vs. Alcoholic $B_{max} = 335 \pm 45$ fmol/mg, $P < 0.05$) and α1C subunit expression (Alcoholic $2^{-\Delta\Delta Ct}1.69 \pm 0.03$, Control $2^{-\Delta\Delta Ct}0.96 \pm 0.02$, $P < 0.0005$), respectively. This loss of L-type calcium channel activity, accompanied by an increased channel expression with chronic alcohol consumption may be a precipitating factor in alcoholic heart disease, leading to the onset of other adaptive mechanisms and, eventually, the clinical syndrome of heart failure.

**2683-Pos**
**Characterization of the Calmodulin-Binding Site in the N Terminus of $Ca_V1.2$**
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$Ca_V1.2$ is an L-type $Ca^{2+}$ channel from a family of voltage dependent $Ca^{2+}$ channels(VDCC)distributed mainly in cardiac and smooth muscle, endocrine cells and neurons, which produce calcium influx in response to membrane depolarization. Interaction of calmodulin (CaM) with the C-terminus (CT) of the L-type $Ca_V1.2$ channel is crucial for $Ca^{2+}$-dependent inactivation (CDI). CaM also binds to the N-terminus (NT), and a CaM-formed "bridge" between CT and NT has been proposed to control CDI.
We characterized the interaction of CaM with its NT-binding peptide. Using ITC, we determined the binding of CaM to the NT-binding site is $Ca^{2+}$-dependent with an affinity of 0.6 μM. The $Ca^{2+}$ dependence of the NT-CaM interaction makes it a plausible candidate for a reversible, $Ca^{2+}$/CaM-dependent regulatory process such as CDI. However, our results do not support a model in which CaM forms a direct "bridge" between the N and C-terminal CaM binding sites. NSCaTE (N-terminal spatial $Ca^{2+}$ transforming element), which appears to play a substantial role in CDI of CaV1.3, does not appear to be strongly involved in the inactivation process in $Ca_V1.2$. Mutations in NT of $Ca_V1.2$ that abolished the binding of CaM only slightly weakened the CDI but also accelerated the VDI. CaM did not foster an interaction between the CaM-binding peptides of NT and CT. Thus, the role of CaM's interaction with the $Ca_V1.2$ NT remains to be determined.

**2684-Pos**
**The L-Type Calcium Channel C-Terminus is a Mobile Domain that Competes with Calmodulin Modulation of Calcium Current**
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The L-type Ca channel (CaV1.2) distal carboxyl-terminus (CCt) has multiple functions. CCt inhibits L-type calcium current (ICa,L), and is a mobile element that translocates to the nucleus where it regulates CaV1.2 transcription. CCt

interacts with CaV1.2 in a similar domain as calmodulin (CaM). The purpose of this study is test the hypothesis that CaM and CCt compete for functional interaction with CaV1.2. ICa,L and barium current (IBa,L) was recorded from HEK 293 cells transfected with CaV1.2 + CaVbeta2a. This background was compared to cells additionally transfected with CaM and/or CCt. The CaV1.2 expressed was deleted at position 1733 (numbering based on rabbit sequence), and CCt corresponded to amino acids 1821-2171. ICa,L and IBa,L was recorded in each cell, and we compared the increase of current, the shift of activation midpoint, and current kinetics of ICa,L versus IBa,L within a given cell. Maximal conductance ratio Ca/Ba is ~0.4 for CaV1.2+CaVbeta2a expression. Addition of CaM co-expression does not alter Ca /Ba conductance. CCt co-expression significantly increases the relative Ca /Ba ratio 2-fold, and this effect is reversed by CCt+CaM co-expression. Examination of the peak I(V) curves suggests that midpoint of activation was not affected, and ICa,L density is not different in for all transfection conditions. We conclude that CCt attenuation of conductance occurs only with Ba, and is consistent with a Ca alleviation of CCt block. Thus, CaM and Ca functionally compete to limit CCt auto-inhibition of CaV1.2 current.

**2685-Pos**

**Calmodulin Release from the IQ Domain of $Ca_v1.3$ Channels During Calcium Dependent Inactivation?**

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Calmodulin (CaM) regulation of $Ca_V$ channels has long fascinated biophysicists, with structure-function analysis mostly focused on an IQ domain in the carboxy-terminus of channels. It is clear that $Ca^{2+}$-free CaM (apoCaM) preassociates with the IQ domain before $Ca^{2+}$ entry through channels (see Liu X *et al* this meeting), and that $Ca^{2+}$/CaM has the potential to bind the IQ. Hence, $Ca^{2+}$-dependent inactivation (CDI) of channels has been thought to result by transducing $Ca^{2+}$-dependent conformational changes of CaM, all while bound to the IQ. By contrast, functional analysis of our structure of $Ca^{2+}$/CaM complexed with the IQ domain of $Ca_V2.1$ (Structure **16**:607) hints that $Ca^{2+}$/CaM may *depart* from the IQ domain during channel regulation. To generalize this hypothesis, we here alanine scanned the entire IQ domain of $Ca_V1.3$, an exemplary prototype of $Ca_V1$ channels with high homology to long-studied $Ca_V1.2$. Importantly, alanine substitution likely preserves backbone fold, whereas prior studies often used more disruptive mutations targeting specific loci. As before, alanine substitution of the signature isoleucine strongly suppressed CDI. Suprisingly, however, CDI was strongly diminished only at one other residue, *upstream* of the central isoleucine. Altering many other sites, presumed important for $Ca^{2+}$/CaM-IQ binding in crystal structures, left CDI unscathed. Moreover, we homology modeled $Ca^{2+}$/CaM bound to the $Ca_V1.3$ IQ domain, based on x-ray structures of $Ca_V1.2$. We then computed (*Robetta*) the energetic cost of alanine mutations ($\Delta\Delta G$ for binding). If $Ca^{2+}$/CaM-IQ binding begets CDI, plots of CDI versus $\Delta\Delta G$ should define a Boltzmann function. Instead, a highly scattered relationship was produced. Thus, CDI may involve (partial) departure of $Ca^{2+}$/CaM from the IQ domain of $Ca_V1.3$, to interact with alternative sites (Yang, Ben Johny, & Yue, this meeting). This departure would fundamentally transform our understanding of CaM/channel regulation.

**2686-Pos**

**Structural and Functional Comparison Between the Effects of CaBP1 and Calmodulin on the Voltage-Gated Calcium Channel Cav1.2**

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Calcium-dependent feedback modulation is central to voltage-gated calcium channel (CaV) function. The bilobed EF-hand containing calcium-binding protein 1 (CaBP1) is thought to modulate CaV function by competing with the homologous calmodulin (CaM) for binding to the CaV C-terminal IQ-domain. In CaV1.2, CaBP1 inhibits calcium-dependent inactivation (CDI) of CaV1.2 and introduces calcium-dependent facilitation (CDF). We investigated the origins of functionally important differences between CaM and CaBP1 by creating a number of CaM/CaBP1 chimeras and found a clear division of function between the various elements. Determination of the CaBP1 X-ray structure revealed an interaction site between linker and one of the lobes that is required for inhibition of CaV1.2 CDI. Using titration calorimetry, we found that, similar to CaM, the CaBP1 C-terminal lobe is responsible for high-affinity interaction with the IQ-domain. CaM requires functional C-terminal lobe EF-hands for supporting CDI. In contrast, we found that CaBP1 does not require functional EF-hands for inhibition of CDI. CaBP1-mediated CDF however requires all EF-hands to be functional and also has different requirements from CaM-mediated CDF, suggesting that these are two distinct processes. Overall, the data reveal those parts of CaBP1 that set it functionally apart from CaM and provide a framework for understanding how CaBP1 and CaM regulate CDI and CDF on CaV1.2.

## Voltage-gated K Channels-Gating III

**2687-Pos**

***In-Silico* Activation and Deactivation of the Pore Domains of Voltage- and Ligand-Gated Ion Channels**

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Several homotetrameric ion channels have been crystallized in either the open or closed state. Here we used Monte Carlo-energy minimizations and the program ZMM, which takes advantage of channel symmetry, to simulate activation/deactivation of the pore-forming domains of four channels and estimate their state-dependent energetics. Deactivation of the open-Kv1.2 x-ray structure by a counter-clockwise torque (intracellular view) applied to four S5 N-ends yielded a conformation with S5/S6 alpha-carbons RMS-deviating by 1.8 Å from the closed-channel model (Pathak et al., 2007). The data that cadmium binds to the closed *Shaker* with engineered cysteines in S6s (del Camino et al., 2005) is rationalized in our model with two cadmium ions neutralizing the four ionized cysteines (Bruhova and Zhorov, 2005). Reactivation of our deactivated-Kv1.2 model by the clockwise torque yielded a conformation that RMS-deviates from the open-Kv1.2 structure by 1.4 Å. Using the same approach we deactivated and reactivated the pore-domain of KvAP (truncated to match the KcsA-sequence length) and arrived at models with RMSDs of 2.0 and 1.9 Å from the KcsA and KvAP x-ray structures, respectively. Activation and deactivation of pH-gated KcsA with centrifugal and centripetal forces, respectively, applied to M2 C-ends (Tikhonov and Zhorov, 2004) yielded conformations that RMS-deviate from the x-ray structures of MthK and KcsA by $<$ 2.0 Å. Using the same forces, we activated and deactivated the cyclic-nucleotide gated NaK channel (truncated to match the open-NaK sequence length) and arrived at models with RMSDs $\leq$ 1.5 Å from the closed and open x-ray structures, respectively. In our models, the pore domains of the voltage- and ligand-gated channels are most stable in the open and closed states, respectively, suggesting an intrinsic instability of the pore domains in non-crystallized states. Supported by CIHR and NSERC.

**2688-Pos**

**All-Atom Molecular Dynamics Simulations of the K+ Channel Chimera Kv1.2/Kv2.1**

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Voltage-gated $K^+$ channels (Kv) are composed of four subunits, each of which contains six trans-membrane domains (TMs), S1 through S6. The S1-S4 segments comprise the voltage-sensing domain (VS), which senses membrane potential and controls the gating of the pore domain (PD). Although still controversial, the voltage-sensing domain undergoes conformational changes within the membrane electric field, upon membrane depolarization, that is mechanically transferred via the S4-S5 linker to the intracellular gate of the channel. MD simulations of portions of PD and VS regions highlighted the importance of their flexibility for proper channel function. Nevertheless, a comprehensive description of the dynamics of both domains at atomic level has not been provided yet. Here we report the analysis of all-atom multiple molecular dynamics simulations (~200 ns) of the entire Kv1.2/2.1 chimera, consisting of the $\alpha$ and $\beta$ chain embedded in a 549 monomer POPC bilayer, and immersed in a box of 135K explicit SPC water molecules at 300 K. We used principal components analysis (PCA) of the C$\alpha$ atomic fluctuations covariance matrix to analyze the essential subspace that characterizes the channel internal dynamics. Briefly, we observed an up to 4.5 Å conformational drift of VS from its starting position. The average RMSF of the S3b-S4 domain was between 1.6 and 3.0 Å. Relative to the pore region, i) the second principal component shows that all four VS domains fluctuate in a concerted manner and affect the flexibility of the intracellular gates; ii) the first principal component reveals that T1 domain moves approximately 3.5 Å downwards, influencing the local structure and dynamics of the neighboring intracellular gate. Protein-lipids interactions are crucial for channel structure/function. Thus, the contributions of H-bonds and salt-bridges between channel atoms and lipid head-groups on global channel dynamics will be illustrated.