cell patch-clamp technique and Ca^{2+} signals and ROS production were measured with the fluorescent probes, Fluo 3-AM and CM-H_2DCFDA, respectively. The levels of the α_{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 α_{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 α_{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.
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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 ± 21nM vs. Alcoholic, 260 ± 18 nM) or contraction (Control, 11.9 ± 0.6 μm, Alcoholic,12.7 ± 1.2 μm. However, a blunted inotropic response (increase over basal: Control, 90 ± 19% vs. Alcoholic 39 ± 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