a novel oxygen sensing mechanism of cardiac L-type Ca channels which is independent of mitochondrial ROS and is partially regulated by PKA phosphorylation in the left ventricle. When oxygen pressure was locally decreased from 150 to 5 mmHg within 50 ms, an immediate suppression (25%) occurred in baseline I_{ca} that maximized in 40-50 seconds. This response was inhibited by PKA phosphorylation on the left but not the right ventricle. Inhibiting Ca dependent inactivation using Ba^{2+} as the charge carrier, lead to 40% suppression of I_{Ba} within the first 5-15s of exposure. This effect was independent of PKA phosphorylation and equally affected both ventricles. Inhibiting SR Ca release with 5uM thapsigargin did not mimic the response seen with Ba^{2+}. However, inhibiting Calmodulin using CaM inhibitory peptide 290-309 partially suppressed phosphorylated I_{ca} in the left ventricle. This effect was also present in HEK 293 cells expressing all subunits of the recombinant L-type Ca channel. Furthermore, mutating 80 amino acids in the Ca binding/IQ domain of the alpha 1C subunit which removes Ca dependent inactivation and leads to similar kinetics of I_{Ba} and I_{Ca}, abolished the suppression of I_{Ba} under low O_2. Based on these observations we propose that Cardiac L-type channels have oxygen sensing properties and that Ca/Calmodulin binding domain is a key site in this process.

940-Pos Board B819

Calcium channels regulate myocardial compaction

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BACKGROUND: Calcium regulation is important for cardiac myocyte function and cardiac development. For example, calcium channel blockade and deletion of calcium-regulatory proteins leads to abnormal cardiac morphogenesis. We have previously demonstrated that deletion of the major calcium channel isoform (CaV1.2) from the developing heart leads to grossly normal cardiac structure, but late embryonic demise.
OBJECTIVE: Determine the effects of calcium channel deletion on cardiac development.
METHODS: Global deletion of the major cardiac LTCC isoform, CaV1.2, was obtained using floxed-CaV1.2 mice (Dr. Franz Hofmann) mated to beta-actin-Cre mice. Conditional deletion of CaV1.2 in the AHF was obtained using Mef2c-AHF-Cre mice (Dr. Brian Black). Specimens at various stages of gestation were examined by embryonic echocardiography and cardiac heart rates and left and right ventricular shortening fractions were quantified. Embryos were harvested and examined for changes in gross morphology and cardiac morphology by visual observation and histological methods. Compaction of the ventricular myocardium was quantified on histologic sections.
RESULTS: Embryos containing global or conditional deletions of CaV1.2 die at E14 and E15, respectively. Approximately 1 day prior death, null embryos had abnormal cardiac function with depressed shortening fraction and abnormal heart rates. Over the next day, cardiac failure became apparent, with evidence of pericardial effusions and body wall edema. Approximately 2 days prior to death, the ventricular myocardium appeared to lack compaction of the trabeculae into the compact layer of the myocardium. Prior to this, the embryonic hearts appeared normal.
CONCLUSIONS: The presence of normal calcium channels is important for late maturation of the embryonic ventricular myocardium. Deletion of CaV1.2 caused ventricular non-compaction followed by depressed cardiac function, heart failure, and late embryonic demise.

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Enhancement of the Cav3.1 Channel Activity by PKA in Ventricular Myocytes of a1G Transgenic Mice

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Low voltage-activated T-type Ca^{2+} channels (Cav3 or TTCC) play an important role in regulating the pacemaker activities in the heart. Since adrenergic system is critical for heart rate regulation and the TTCC is involved in cardiac rhythmn generation, it is important to examine the regulation of the TTCC by the adrenergic-PKA system. In this study, we sought to resolve the question whether Cav3.1 in cardiac myocytes is regulated by PKA. **Methods:** Cav3.1 α1G transgenic mice were established with the cardiac specific and inducible system engineered by the Robbins group. Whole cell voltage clamp was used to measure the $I_{Ca\text{-}T}$ before and after isoproterenol application. $I_{Ca\text{-}T}$ was also recorded with or without cAMP (10μM) in the pipet. **Results**: (1) There is robust $I_{Ca\text{-}T}$ (25.3 ± 12.5pA/pF, n=13) in ventricular myocytes isolated from α1G TG mice but no $I_{Ca\text{-}T}$ was observed in ventricular myocytes from control mice; (2) $I_{Ca\text{-}T}$ in α1G TG myocytes was significantly increased by isoproterenol application (before vs. after: 15.0 ± 3.3pA/pF, n=4 vs. 11.7 ± 4.7pA/pF, n=4, at -40mV, $p<0.05$). This indicates Cav3.1 channel activity was probably up-regulated by isoproterenol-activated PKA in myocytes isolated from adult α1G transgenic mice. (3) cAMP can greatly increase both T-type (with vs. without cAMP: 48.9 ± 29.2pA/pF, n=4 vs. 25.3 ± 12.5pA/pF, n=13, maximal $I_{Ca\text{-}T}$, $p<0.05$) and L- type calcium currents (with vs. without cAMP: 19.9 ± 7.4pA/pF, n=4 vs. 7.6 ± 3.7pA/pF, n=13, maximal $I_{Ca\text{-}L}$, $p<0.05$). This further confirms the up-regulation effect of PKA on the Cav3.1 channel activity**. Conclusions:** For the first time, we found that PKA activation enhances Cav3.1 channel activity in ventricular myocytes of mice. This finding may shed a light on the physiological and pathophysiological (arrhythmogenic) effects of sympathetic regulation of pacemaker activities in the heart through T-type calcium channels.

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Cardiovascular profile of newly developed Diltiazem analogs

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Many diltiazem related L-VDCC blockers were developed using a multidisciplinary approach. This current study was to investigate and compare diltiazem with to the newly developed compounds by mouse Langendorff-perfused heart, Ca^{2+}-transients and on recombinant L-VDCC. Five particular compounds were selected by the ligand-based virtual screening procedure (LBVS) (5B, M2, M7, M8 and P1). Wild-type human heart and rabbit lung α_1 subunits were expressed (combined with the regulatory $\alpha_2\delta$ and β_3 subunits) in *Xenopus leavis* oocytes using a two-electrode voltage clamp technique. Diltiazem is a benzothiazepine Ca^{2+} channel blocker used clinically for its antihypertensive and antiarrhythmic effects. Previous radioligand binding assays revealed a complex interaction with the benzothiazepine binding sites for M2, M7 and M8. (*Carosati E. et al. J. Med Chem. 2006, 49; 5206*). In agreement with this, the relative order of increased rates of contraction and relaxation at lower concentrations ($<10^{-6}$M) in un-paced hearts was M7>M2>M8>P1. Similar increases in Ca^{2+} transients were observed in cardiomyocytes. Diltiazem showed negative inotropic effects whereas 5B had no significant effect. Diltiazem blocks Ca^{2+} currents in a use-dependent manner and facilitates the channel by accelerating the inactivation and decelerating the recovery from inactivation. In contrast to diltiazem, the new analogs had no pronounced use-dependence. Application of 100 μM M8 and M2 showed ~10% tonic block, shifted the steady-state inactivation in hyperpolarized direction and the current inactivation time was significantly decreased compared with control (219.6 ± 11.5 ms, 226 ± 14.5 vs. 269 ± 12.9 ms). Contrary to diltiazem, the recovery from the block by M8 and M2 was comparable to control. All of the compounds displayed the same sensitivity on the Ca^{2+} channel rabbit lung α_1 except P1. Taken together, these findings suggest that M8 and M2 might directly decrease the binding affinity or allow more rapid dissociation from the benzothiazepine binding site.

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Charge-dependent And Isoform-specific Interactions Between ProTxii And T-type Calcium Channels

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ProTxII, peptide toxin isolated from the venom of the tarantula Thrixopelma puriens, modifies voltage-dependent activation of both T-type calcium (Ca) channels and voltage-gated sodium (Na) channels. In the presence of ProTxII (5μM) the voltage at half maximal activation (V1/2) of the CaV3.1 isoform is shifted positive (>25mV) and maximum conductance (Gmax) decreases (~50%). Interestingly, the toxin's effects on this channel were completely precluded in the presence of high extracellular divalent concentrations indicating a role for surface charge-like, electrostatic interactions with the channel. Several mutant toxins in which individual basic residues were neutralized were tested for activity on CaV3.1. Three of these mutants, R13Q, R22A, and K28A, significantly disrupted the ability of the toxin to both shift channel activation and decrease Gmax. Two other mutations: K4Q and K14A, showed minimal or no effect, thus indicating an important yet specific role of charge in ProTxII's interaction with CaV3.1. The gating kinetics of T-type Ca channels varies among the three known isoforms suggesting there might be differences in the gating structures and, therefore, potential gating modifier toxin interaction surfaces as well. In CaV3.3, 5μM ProTxII reduced Gmax by approximately 60%, similar to what was seen for CaV3.1. However, unlike in CaV3.1, this concentration of toxin produced only a minimal shift in voltage dependent activation (2mV). These results suggest significant differences in the extracellular surface of T-type Ca channels across isoforms, particularly in terms of surface charge distribution close to one or more of the channels' voltage sensors.

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Modeling L-type Calcium Channel with Dihydropyridines

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