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.

941-Pos Board B820

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.

942-Pos Board B821

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.

943-Pos Board B822

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.

944-Pos Board B823

Modeling L-type Calcium Channel with Dihydropyridines

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Dyhydropyridines (DHPs) is a major class of L-type calcium channel (LCC) ligands, which have boat-like six-membered ring with NH-group at the stern, aromatic moiety at the bow, and various substituents at the port and starboard sides. DHPs demonstrate antagonistic or agonistic action, which was previously explained as stabilization or destabilization, respectively, of the closed-gate state by the hydrophilic or hydrophobic port-side substituent. Here we used Monte Carlo energy-minimizations to dock various DHPs in the open-LCC model (Tikhonov & Zhorov, 2008). The calculations suggest a novel structural model in which agonistic and antagonistic actions are determined by different parts of the DHP molecule and have different molecular mechanisms. In our model, DHP polar moieties at the stern, bow, and starboard form H-bonds with side chains of Tyr_IVS6, Tyr_IIIS6, and Gln_IIIS5, respectively. The aromatic moiety at the bow binds to Phe_IIIP. We propose that these contacts with the well-known DHP-sensing residues stabilize the channel's open-gate conformation. Since these contacts are common for various DHPs, our model explains why both agonist and antagonists increase probability of the long-lasting channel openings and why even partial disruption of the contacts eliminates the agonistic action. In our model, the port-side is exposed to the permeation pathway and approaches the selectivity filter. Hydrophobic ports-side group of antagonists may induce long-lasting channel closings by destabilizing calcium coordination with the selectivity-filter glutamates in domains III and IV. In contrast, agonists, which have either hydrophilic or no substituent at the port-side, lack this destabilizing effect. Our model explains action of DHPs with diverse substituents. Thus, long substituents at the port-side are readily accommodated in the pore. Long substituents at the starboard-side protrude in the III/IV domain interface, explaining activity of DHPs linked to a permanently charged group. Supported by CIHR.

945-Pos Board B824

Reinterpretation of SCAM Data in View of Kv1.2-based Models of MTSET-Substituted CaV2.1 Channels

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Molecular modeling of calcium channels, which are important drug targets, relies on sequence alignments with potassium channels. Huber et al. (2000) and Zhorov et al. (2001) proposed alignments for outer and inner helices, respectively. Zhen et al. (2005) attempted to identify pore-lining residues in CaV2.1 channel using the substituted-cysteine accessibility method (SCAM) and interpreted their data as inconsistent with known sequence alignments. Indeed, the inner-helix residues in positions *i15*, *i18*, *i19*, and *i22* face the Kv1.2 pore. In agreement with this, 2-(trimethylammonium)ethyl methanethiosulfonate (MTSET) applications to CaV2.1 with engineered cysteines in positions *2i18* (domain 2, inner-helix position 18)*, 4i18,* and positions *i15* and *i19* of all four domains decreased current. However, CaV2.1 with cysteine substitutions in positions *1i18*, *3i18* and position *i22* of all four domains were not blocked by MTSET. Furthermore, despite the outer-helix positions *2o10* and *4o10* are far from the pore, corresponding cysteine substitutions were blocked by MTSET. Here we created the Kv1.2-based model of CaV2.1 using the above alignments. In this model, engineered cysteines in positions *i22* are surrounded by large hydrophobic residues, which would preclude cysteine ionization and hence reaction with MTSET. We further created CaV2.1 models with MTSET-substitutions in other positions and used Monte Carlo-energy minimizations to find energetically optimal conformations. The ammonium group of MTSET in positions *2i18* and *4i18* occludes the inner pore, while in positions *1i18* and *3i18* it protrudes in the domain interface. The ammonium group of MTSET in positions *2o10* and *4o10* approaches the pore, being closer to the pore axis than that in positions *1i18* and *3i18*. Thus, our model reinterprets experiments of Zhen et al. (2005), validates the above alignments, and suggests a similar folding of voltage-gated potassium and calcium channels. Supported by CIHR.

946-Pos Board B825

Modeling L-type Calcium Channel with Phenylalkylamines

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Phenylalkylamines (PAAs), a major class of L-type calcium channel (LCC) blockers, have aromatic rings A and B connected by a flexible chain with cyano and ammonium groups proximal to rings A and B, respectively. Structural aspects of ligand-channel interactions remain unclear. We have built LCC models as in (Tikhonov and Zhorov, 2008) with KvAP, MthK, Kv1.2 and KcsA as templates and used Monte Carlo energy-minimizations to dock devapamil, verapamil, and gallopamil, which have three, four, and five methoxy groups, respectively. The PAA-LCC models have the following common features: meta-methoxy group in ring A accepts an H-bond from Y1179(3i10) in domain 3 inner-helix position 10, the ammonium group is stabilized at the focus of P-helices, and the cyano group coordinates a Ca2+ ion bound to the selectivity-filter glutamates in domains 3 and 4. The latter feature can explain the well-known effect of Ca2+ potentiation of PAA action. Our models are also consistent with structure-activity and mutational studies. For instance, mutation of Y1490(4i11) affects action of devapamil, but not verapamil and gallopamil (Johnson et al., 1996). In our models, the single meta-methoxy group in ring B of devapamil accepts an H-bond from Y1490(4i11), while meta- and para-methoxy groups in ring B of verapamil and gallopamil chelate the Ca2+ ion. Mutation T1066(3o14)Y in domain 3 outer-helix position 14 enhances action of devapamil and verapamil, but not gallopamil (Huber et al., 2004). Our models predict that para-methoxy group in ring A of devapamil and verapamil accepts an H-bond from Y3o14, while tri-methoxylated ring A of gallopamil is too bulky to approach Y3o14. Docking of devapamil in different models shows that the Kv1.2 template is most consistent with the experimental data. The closed (KcsA-based) model has the same ligand-channel contacts, but with weaker interaction energy. Supported by CIHR.

947-Pos Board B826

Effect Of $Ca_V\beta$ Subunits On Structural Organization Of $Ca_V1.2$ Calcium Channels As Revealed By Three-color Fret Microscopy

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Voltage-gated $Ca_v1.2$ calcium channels play a crucial role in Ca^{2+} signaling. The poreforming α_{1C} subunit is regulated by accessory $Ca_v\beta$ and $\alpha_2\delta$ subunits. $Ca_v\beta$'s are cytoplasmic proteins of various size encoded by four different genes ($Ca_v\beta_1$ - β_4). Here we investigated the effect of three major $Ca_v\beta$ types, β_{1b}, β_{2d} and β_3, on the structure of $Ca_v1.2$ by measuring inter and intramolecular distances between α_{1C} and β in the plasma membrane of COS1 cells using three-color FRET microscopy. The results show that $Ca_v1.2$ channels are in close proximity in the plasma membrane. The presence of different $Ca_v\beta$'s does not result in significant differences in intramolecular distance between the termini of α_{1C}, but significantly affects intermolecular distance between the termini of neighbor α_{1C} subunits, which varies from 67 Å (β_{1b}) to 79 Å (β_3). Thus, our results show conclusively that plasma-membrane density of $Ca_v1.2$ channels depends on the type of $Ca_v\beta$'s present, suggesting a possible mechanism contributing to differences in Ca^{2+} signaling between various cell types.

948-Pos Board B827

Quantification Of L-type Ca Current Inactivation Mechanisms In Trout Ventricular Myocytes.

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Inactivation of L-type calcium current (I_{Ca}) is due to two mechanisms: voltage-dependent inactivation (VDI) and calcium-dependent inactivation (CDI). In fish cardiac myocytes, it is unknown whether Ca release from the sarcoplasmic reticulum (SR) participates in CDI of I_{Ca}. This study assesses the relative contribution of different inactivation mechanisms of I_{Ca} in trout ventricular myocytes. Trout ventricular myocytes were enzymatically isolated. I_{Ca} was recorded using whole cell patch clamp with Na- and K-free solutions to avoid contaminating currents. With a low concentration of a slow Ca buffer (EGTA 2mM) in the pipette solution, I_{Ca} inactivated slowly (compared to mammalian cardiac myocytes): the time to reach 37% of peak current (T_{37}) was 26.2 ± 2.4 ms (mean $\pm$ SEM, n=14). When a fast Ca buffer (BAPTA 10 mM) was present in the pipette solution I_{Ca} decay was similar to the decay in the presence of EGTA (T_{37}: 25.4 ± 1.5 ms, NS, t-test, n=9). When barium was used as a charge carrier, I_{Ba} inactivates mainly via VDI and T_{37} was significantly increased (43.7 ± 3.1 ms, n=9, $p<0.05$, t-test), albeit T_{37} is twice faster than in mammalian cardiac myocytes. We quantified the relative contribution of VDI and CDI according to the method previously described [Brette *et al.* (2004). *Circ Res;* **95;** e1-7]. We measured the fraction of current remaining 20 ms after its peak (I_{R20}). I_{R20} was 0.39 ± 0.03 in EGTA, 0.42 ± 0.02 in BAPTA and 0.64 ± 0.03 in barium. We estimated that CDI represents ~39% of total fast I_{Ca} inactivation, and that SR Ca release causes only ~12% of CDI. We conclude that the main inactivation mechanisms in the trout myocyte are due to VDI and CDI from Ca entering the cell via I_{Ca} and not SR Ca release. *Supported by the Wellcome Trust and the BBSRC.*

949-Pos Board B828

Egg Coat Proteins Activate Ca^{2+} Entry into Mouse Sperm via CATSPER Channels

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During mammalian fertilization, the contact between sperm and egg triggers increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in sperm. Voltage-gated Ca^{2+} channels (Ca_Vs) are believed to mediate the initial phase of $[Ca^{2+}]_i$ increases in sperm induced by egg coat (zona pellucida, ZP) glycoproteins, while