of a P2X4 receptor based on normal mode analysis and molecular dynamics (MD) simulations. Starting with the first available P2X4 crystal structure in the resting state, a normal mode that couples the motions of three β strands (β1, β13 and β14) at the trimeric interface of the ligand binding domain (LBD) and the motion of the pore-forming helix (TM2) of the transmembrane domain (TMD) was identified. The resulting widening of the fenestrations above the TMD and the opening of the TMD pore are in close agreement with observed signatures of channel activation. Four charged residues implicated in ATP binding are located in β1, β13 and β14. P2X4 activation was further investigated by MD simulations in explicit water. ATP was placed near the putative binding site in two opposite orientations, with the adenine either proximal or distal to the TMD. In simulations with proximal adenine, the adenosine ring inserted between β1 and β14 and the phosphate group moved downward. At the same time β1 and β14 approached each other to close in on the ATP, allowing close interactions with the four charged residues. The motions of these β strands are similar to those in the normal mode putatively representing channel activation. In simulations with distal adenine, the ATP hindered the closure between β1 and β14, perhaps representing a desensitized state.

Our computational studies produced the first complete model, supported by electrophysiological data, for how ATP binding leads to P2X4 channel activation. The detailed gating mechanism will be essential for the rational drug design.

1715-Pos Board B485
Calcium Channel CatSper is a Non-Genomic Progesterone Receptor of Human Sperm
James F. Smith1, Yurry Kirichenko1, Polina V. Lishko1,2
1University of California, San Francisco, San Francisco, CA, USA, 2University of California, Berkeley, Berkeley, CA, USA.

Elevation of intracellular Ca2+ regulates sperm motility, chemotaxis, capacitation and the acrosome reaction, and play a vital role in the ability of the sperm cell to reach and fertilize the egg. In mammalian spermatozoa, the flagellar pH-dependent Ca2+ channel CatSper is the main pathway for calcium entry as measured by the whole-cell patch clamp technique. Mouse CatSper channel is activated by alkaline intracellular pH, but human CatSper requires also a ligand. By applying the patch-clamp technique to mature human spermatozoa, we found that nanomolar concentrations of female hormone progesterone activate CatSper and this action explains the mechanism of non-genomic action of progesterone on human sperm cells. Progesterone is released by cumulus cells surrounding the oocyte and serves as a chemoattractant for human spermatozoa.

Interestingly, human CatSper can be further potentiated by prostaglandins, but apparently through a binding site other than that of progesterone. Behavior of CatSper channel gradually changes during spermiogenesis with CatSper channel being fully operational only in mature spermatozoa. Physiological regulation of CatSper channel by different components of seminal plasma as well as hormones of female reproductive tract will be discussed.

1716-Pos Board B486
Comparative Study of the Bacterial Sodium Channel NaChBac Function using Patch Clamp and A.C. Impedance Spectroscopy in a Tethered Membrane
Bruce A. Cornell1, Gwenaëlle Scolan2, Andrew M. Pow1, Sonia Carmi1, Bonnie A. Wallace3
1Tethered Membranes Pty Ltd, Sydney, Australia, 2Université Joseph Fourier, Grenoble, France, 3University of London, London, United Kingdom.
The tetrameric voltage gated sodium channel NaChBac from Bacillus halodurans was studied by conventional patch clamp techniques in synthetic lipid liposomes composed of dioleoyl phosphatidylcholine, and by a.c. impedance spectroscopy within a family of synthetic tethered palmitoleic phytanyl membranes. By patch clamp techniques the single channel conduction was found to be 11pS ± 1pS in agreement with published values. The same protein when incorporated into tethered membranes from Cymal-5 detergent micelles at concentrations of up to 100 nM NaChBac showed levels of conduction up to ~0.4mS/cm2. Electrode areas of 2 mm2 from Cymal-5 detergent micelles at concentrations of up to 100 nM NaChBac showed a symmetrical conduction with bias potential suggesting an equal like-hood of the NaChBac incorporation in either orientation. In the tethered membranes, however, the orientation dependence and thus voltage dependence may be controlled by the tethered membrane composition. The results for a number of tethered membrane constructs will be presented in which the tether density and spacer lengths are varied. The effects of these variations on the observed results will be discussed and examples given of using tethered membranes to measure Mibefradil and other ligand (drug) binding to NaChBac.

1717-Pos Board B487
The Open Conformation of the Mg2+- Channel CorA from Solvent Accessibility and Distance Constraints
Pornthep Sompornpisut1, Olivier Dalmas1, Eduardo Perozo2
1Chulalongkorn University, Bangkok, Thailand, 2The University of Chicago, Chicago, IL, USA.

Using a combination of electrophysiology and spectroscopic approaches we have recently shown that CorA acts as a Mg2+-gated Mg2+-selective channel. NiEDDA/O2 accessibility together with intersubunit distances were measured in two conditions: 1) Saturating Mg2+, which stabilizes CorA in the closed conformation and 2) The nominal absence of diverant ions, which stabilizes the open state. Here, we use a computational approach for incorporating both solvent accessibility data and distance constraints through restrained molecular dynamics (MD) simulations using CorA in a closed conformation as the starting structure. The accessibility restraints were enforced through interactions between a pseudoatom representation of the spin-label and environmental probe-surrounding particles. Intersubunit Cβ-Cβ distances estimated from DEER experiments, together with the accessibility data were used to generate hundreds of models by varying the upper and lower bound of distance constraints and increasing the number of MD refining cycles. The top 25 models show structural convergence especially around the stalk and inner helices, where the ERP restraints were imposed. After a second round of refinement, the stability of the top ranked structure was evaluated by an all-atom MD simulation in a fully hydrated phospholipid bilayer. After 2ns of the simulation, the RMSD of the stalk and inner helices is stable around 4-5Å. Based on the pore-radius profile, the permeation pathway has dilated enough to allow a hydrated Mg2+, as expected if this conformation is conductive. A linear interpolation between the closed and open conformations suggests a gating mechanism for CorA, where the tips of the stalks come together like the ribs of an umbrella. After a kink, this motion translates into an expansion of the cavity and the mouth of the pore opens up with a motion reminiscent of an iris of a camera.

Cardiac Electrophysiology I

1718-Pos Board B488
Interleukin-1β Modulates Cardiac L-Type Calcium Channel via PKC Signalling Pathways in Mice
Nabil El Khoury, Céline Fiset.
Université de Montréal, Montréal, QC, Canada.
Background- Inflammation is being widely recognised as a key component of heart disease. Several pro-inflammatory cytokines have been shown to mediate heart failure and cardiac remodelling. High levels of cytokines have been reported in patients suffering from arrhythmias suggesting they might be implicated in rhythm disturbances and electrical remodelling. Of these cytokines is interleukin-1β (IL-1β), one of the earliest and main cytokines induced via the inflammatory response. The specific role it plays in modulating cardiac ion channels is largely unknown.

Objective- The L-type calcium current (ICaL) plays a critical role in electrical and contractile properties of the heart, therefore, the purpose of this study was to determine the effect of a chronic IL-1β exposure on ICaL density, its underlying z-subunit Cav1.2 and the mechanisms potentially involved.

Methods & Results- The patch-clamp experiments revealed that the ICaL density (pA/pF) was decreased by 36% after a 24H-treatment of IL-1β at pathophysiological concentration (30pg/mL) in cultured neonatal ventricular myocytes compared to controls (at 0 mV, CTL: −6.2 ± 0.5, n=17 and, IL-1β: −4.0 ± 0.5, n=19, p<0.05). The qPCR experiments revealed no differences in Cav1.2 mRNA expression. Confocal imaging showed a significant increase of ROS levels in IL-1β-treated myocytes, however antioxidant treatments failed to restore ICaL density. Conversely, the PKC inhibitor chelerythrine rescued ICaL (at 0 mV, CTL: −5.6 ± 0.5, n=10 and, IL-1β-Chelerythrine: −6.2 ± 0.7, n=14, p<0.05). The implication of PKC was further demonstrated by using the PKC activator PMA which decreased ICaL similarly to IL-1β treatment.

Conclusion- Overall, this study shows that a pathologically relevant concentration of IL-1β significantly decreases the density of ICaL in cardiomyocytes without affecting Cav1.2 expression. The data suggests that IL-1β mediates its effect via PKC activation.

1719-Pos Board B489
Circulating Amylin Amyloid Oligomers Accumulate in the Heart and Induce Cardiomyocyte Dysfunction in a Rat Model of Type-2 Diabetes
Kaleena Jackson, Sanda Despa, Florin Despa.
University of California, Davis, CA, USA.
Amylin amyloid deposition is generally considered a pancreatic disorder and a hallmark of type-2 diabetes. Recently, amylin amyloids were also found in
failing hearts from obese and type-2 diabetic patients, suggesting a possible contribution to cardiac dysfunction. Here, we investigate the mechanism of cardiac amylin accumulation and consequent effects on myocardial structure and function in rats transgenic for human amylin. In this model, we observed that soluble amylin oligomers are released from the pancreas in the blood and accumulate in the heart. Amylin oligomers attach to the sarcolemma and raises the intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(i\)) leading to myocardy dysfunction. In contrast, rats expressing same level of wild-type, non-amyloidogenic rat amylin showed normal cardiac myocyte structure and function. To test whether the rise of [Ca\(^{2+}\)]\(i\) is an amylin oligomer-mediated effect, we measured Ca\(^{2+}\) transients in intact cardiac myocytes incubated with exogenous human/rat amylin. 50 \(\mu\)M of human amylin, which rapidly forms oligomers, increased substantially the amplitude of cardiac myocyte Ca\(^{2+}\) transients, while same concentration of rat amylin had no significant effects. Passive trans-sarcolemmal Ca\(^{2+}\) leak was substantially larger in myocytes incubated with human amylin vs. control, implying that amylin oligomers alter the structural integrity of the sarcolemma and increase sarcolemmal permeability to Ca\(^{2+}\). In conclusion, our data show that amylin oligomers circulate through the blood, accumulate in the heart, and alter cardiac myocyte function by disrupting Ca\(^{2+}\) homeostasis. The results suggest that circulating amylin oligomers should be targeted for pharmacological interventions to prevent heart dysfunction in patients with obesity and insulin resistance.

1720-Pos Board B490
Mitochondrial ROS Promote Recovery of Single Ischemic Cardiomyocytes upon Reoxygenation after Short Near Anoxia
Vladimir Ganitkevich, Klaus Benndorf. Universitaet Jena, Jena, Germany.

Restoration of the blood flow in the ischemic myocardium can result in a lethal reperfusion injury. Reactive oxygen species (ROS) that are formed in the presence of oxygen are believed to play an important role in the post-ischemic injury. Reperfusion of the myocardium is always accompanied by the reoxygenation. Therefore the effect of reoxygenation alone is unknown. Here, using oxygen clamp on in-chip picochambers we exposed single resting cardiomyocytes to near anoxia (pO\(_2\) < 0.1 mm Hg) and subsequently to normoxia. As cardiomyocytes were trapped in picochambers no reperfusion occurred. This allowed us to study the effects of reoxygenation independent of reperfusion. The mitochondrial membrane potential (\(\Delta\Psi\)) was measured in cardiomyocytes simultaneously with \(I_{\text{KATP}}\) providing a measure for the cytosolic ATP. We show that the reoxygenation of ischemic cardiomyocytes alone is insufficient to induce a high ATP turnover rate but that the reperfusion is required for it. Upon reoxygenation to normoxia \(\Delta\Psi\) transiently (6.0 ± 0.7 \(s\)) undershoots the pre-ischemic level. The post-anoxic undershoot of \(\Delta\Psi\) was associated with the increased rate of ATP synthesis, indicating that it was not due to substrate limitation of the F0F1-ATPase. It persisted in cells with stimulated (FCCP; duration 7 ± 1 \(s\)) and inhibited (rotenone; 6 ± 2 \(s\)) respiration. However, in the presence of antimycin, which is known to promote the formation of ROS, the post-anoxic undershoot of \(\Delta\Psi\) was prolonged to 28 ± 3 \(s\). ATP synthesis was also promoted. We propose that ROS burst after reoxygenation from near anoxia by donating electrons to complex IV essentially contributes to a transient stimulation of respiration and promote the post-anoxic recovery of cellular ATP.

1721-Pos Board B491
Optical Mapping of Cardiac ATP Sensitive Potassium Channel Function under Metabolic Inhibition
Keita Uchida\(^1\), Alexey Glukhov\(^2\), Hai Xia Zhang\(^1\), Matt Sulkin\(^2\), Jacob Laughner\(^2\), Geran Kostecki\(^2\), Igor Efimov\(^2\), Colin Nichols\(^1\).
\(^1\)Washington University School of Medicine; Department of Cell Biology and Physiology and Center for the Investigation of Membrane Excitability Diseases, St. Louis, MO, USA. \(^2\)Washington University in St. Louis, St. Louis, MO, USA.

ATP-sensitive potassium channel (K\(_{\text{ATP}}\)) activation can drastically shorten the increased rate of ATP synthesis, indicating that it was not due to substrate pre-ischemic level. The post-anoxic undershoot of [Ca\(^{2+}\)]\(i\) transiently (6.0 ± 0.7 \(s\)) undershoots the pre-ischemic level. The post-anoxic undershoot of [Ca\(^{2+}\)]\(i\) was associated with the increased rate of ATP synthesis, indicating that it was not due to substrate limitation of the F0F1-ATPase. It persisted in cells with stimulated (FCCP; duration 7 ± 1 \(s\)) and inhibited (rotenone; 6 ± 2 \(s\)) respiration. However, in the presence of antimycin, which is known to promote the formation of ROS, the post-anoxic undershoot of [Ca\(^{2+}\)]\(i\) was prolonged to 28 ± 3 \(s\). ATP synthesis was also promoted. We propose that the ROS burst after reoxygenation from near anoxia by donating electrons to complex IV essentially contributes to a transient stimulation of respiration and promote the post-anoxic recovery of cellular ATP.

1722-Pos Board B492
Sulfonylurea Receptor Subunit Composition of K\(_{\text{ATP}}\) Channels in Dog and Human Hearts
Haixia Zhang, Yu-Wen Lin, John W. Verbsky, Angela D. Sellitto, Keita Uchida, Deborah Janks, Urvi S. Lee, Evelyn M. Kanter, Kathryn A. Yamada, Igor R. Efimov, Jennifer S.S. Lawton, Richard B. Schuessler, Colin G. Nichols. Washington University in St. Louis, St. Louis, MO, USA.

ATP sensitive potassium (K\(_{\text{ATP}}\)) channels are assembled by four pore-forming inward rectifier subunits (Kir6.1 or Kir6.2), each associated with one sulfonylurea subunit (SUR1 or SUR2). The differential subunit composition defines distinct K\(_{\text{ATP}}\) channel properties and tissue-specific function. Although the consensus has been that cardiac K\(_{\text{ATP}}\) channels are SUR2A-based channels, our recent studies show that mouse cardiac atrial K\(_{\text{ATP}}\) channels are chamber-specific: SUR1-/SUR2A-based channels in atria vs. SUR2A-based channels in ventricles. To test whether chamber specificity is an universal property of cardiac K\(_{\text{ATP}}\) channels in other species, whole-cell and inside-out excised patch-clamp techniques were applied to examine the effects of SUR- specific channel openers (pinacidil acts on SUR2A and diazoxide on SUR1) on isolated dog and human cardio myocytes. In dog atria, pinacidil- and diazoxide-induced whole-cell currents were 40 ± 4 \(pA/pF\) (n = 14) and 13 ± 6 \(pA/pF\) (n = 11; \(p<0.05\)), respectively; while the ventricular currents were 52 ± 15 \(pA/pF\) (n = 7) and 7 ± 4 \(pA/pF\) (n = 5; \(p<0.05\)), respectively. Also, diazoxide-induced patch current is only 10% of pinacidil-induced current in dog atria and ~16% in ventricles. However, in human atria, pinacidil- and diazoxide-induced whole-cell currents were 90 ± 15 \(pA/pF\) (n = 15) and 78 ± 13 \(pA/pF\) (n = 12; \(p<0.05\)), respectively, while the ventricular currents were 117 ± 57 \(pA/pF\) (n = 3) and 92 (n = 1), respectively. The results indicate that SUR2A-based channels are predominant in dog heart, while abundant SUR1- and SUR2A-based channels co-exist in human heart, in both atria and ventricles. Therefore, there is marked variability between all three species studied, which should be considered carefully when using either mice or dogs to model the effects of human channels in either normal or diseased human hearts.

1723-Pos Board B493
Transient Outward K\(^{+}\) Current (I\(_{\text{to}}\)) is Reduced Especially in the Subendocardium Downregulation of Kv4.2 and KChIP2 Gene Expression in Obese Type-2 Diabetic Rat Hearts
Tatsuya Sato\(^1\), Takeshi Kobayashi\(^1\), Atsushi Kuno\(^1\), Takayuki Mikito\(^1\), Masaya Tanno\(^1\), Tetsuji Miura\(^2\), Noritugu Tohse\(^1\).
\(^1\)Department of Cellular Physiology and Signal Transduction, Sapporo Medical University, Sapporo, Japan. \(^2\)Second Department of Internal Medicine, Sapporo Medical University, Sapporo, Japan.

Background: Cardiac channel remodeling in type 2 diabetes mellitus (T2DM) remains far less understood than in type 1 DM (T1DM). Here, we examined if and how cardiac ion channels were modified by T2DM using OLETF, a rat model of obese T2DM. Methods and Results: We isolated left ventricular (LV) cardiomyocytes from epicardial (EPI) and endocardial (END) regions for whole-cell patch clamp. Action potential duration at 50% repolarization (APD\(_{50}\)) was 1 Hz was significantly longer in OLETF than in non-diabetic control (LET0) (EPI; 8.89 ± 0.69 ms, END; 22.01 ± 3.64 ms vs. 9.89 ± 1.74 ms). Resting membrane potential and membrane capacitance were similar among all groups. L-type Ca\(^{2+}\) current (\(I_{\text{CaL}}\)) inward rectifier K\(^{+}\) current (\(I_{\text{KIR}}\)) were comparable between OLETF and LET0. On the other hand, in the END but not the EPI cardiomyocytes, transient outward K\(^{+}\) current (\(I_{\text{to}}\)) density at 0.1Hz was significantly decreased in OLETF compared to LET0 (END; 8.77 ± 0.79 vs. 14.50 ± 1.31, \(p<0.01\)). In both EPI and END cardiomyocytes, the fast recovering component of \(I_{\text{to}}\) from inactiviation significantly decreased in OLETF compared to LET0. Quantitative RT-PCR analysis of genes encoding \(I_{\text{to}}\) subunits showed that the mRNA levels of Kv4.2 and KChIP2 were significantly reduced by 71% and 34%, respectively, in the END but not the EPI tissue in OLETF compared to those in LET0. The mRNA levels of the other \(I_{\text{to}}\) subunits were comparable in OLETF and LET0.