showed asymmetrical V_j-gating properties with respect to $V_j = 0$. The macroscopic G_j showed substantial reduction (by 82% at $V_j = 100$mV) when depolarizing Cx50-expressing cell, whereas a moderate reduction (by 61% at $V_j = -100$mV) when hyperpolarizing Cx50-expressing cell. This observation is in agreement with previous finding that Cx50 hemichannel gates when the cell is depolarized. Single Cx50/Cx50Cx36N heterotypic channel displayed a main conductance of ~120pS at low V_j pulses ($\pm$ 20mV), but the conductance rectified with increasing V_j pulses. Cx50/Cx50N9R heterotypic *GJ* channel also exhibited asymmetrical V_j-gating properties at macroscopic level, and single channel recordings indicated a main conductance of ~150pS at $V_j = 20$mV. These results demonstrate that the V_j-gating properties and unitary conductance of Cx50 hemichannels were evidently modified when docking with Cx50-Cx36N or Cx50N9R mutants. These novel properties of heterotypic channels provide insights into the V_j-gating mechanisms of Cx50 *GJ* channels.

542-Pos Board B328

A Human Disease Mutation (D50N) Reveals Insights on the Mechanisms for Ca^{2+} Regulation in Human Connexin26 (hCX26) Hemichannels

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Connexin hemichannels allow the release of small metabolites, such as ATP and glutamate, which play important roles in autocrine/paracrine signaling in a variety of cell types. Exacerbated hemichannel opening, however, leads to loss of electrochemical gradients and metabolites, causing cell death. Control of hemichannel opening is indispensable, and is achieved by physiological extracellular Ca2+, which drastically reduces hemichannel activity. Here, we explore the molecular mechanism of Ca2+ regulation in hCx26 hemichannels. We found that outward and tail currents are greatly increased with reduction of external Ca2+. The data show that deactivation kinetics are accelerated as a function of Ca2+ concentration suggesting that Ca2+ facilitates closing of the channels. In addition, we found that an Asp to Asn mutation at position 50 (D50N) - a human mutation that causes disease - has lower apparent affinity for Ca2+ compared with wild-type. Strikingly, unlike wild-type channels, the deactivation kinetics of D50N channels were unaffected by changes in Ca2+. To explore the role of Ca2+ interactions with a negatively charged residue at position 50, we substituted this position by a cysteine residue (D50C) and performed chemical modification with positively or negatively charged methanethiosulfonate (MTS) reagents. The D50C mutant hemichannels display similar properties to those of D50N mutants. Chemical modification of D50C with a negatively charged reagent, MTSES, causes the channels to largely return to wild-type sensitivity to Ca2+. Conversely, positively charged MTS reagents have no effect. In the crystal structure of hCx26 channels, position D50 faces the pore lumen and forms an inter-subunit salt bridge interaction with position K61. Because this interaction appears to take place in open conformation, we propose that Ca2+ facilitates the closing of hCx26 hemichannels by disrupting this salt bridge.

543-Pos Board B329

Identification of Additional Calmodulin Binding Regions in Connexin43

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Gap junction, allowing the intercellular transmission of molecules through its specialized cell membrane channels, plays a major role in intercellular calcium signaling between adjacent cells. Connexin43 (Cx43), the most ubiquitous connexin, belongs to α family of gap junction proteins expressed in heart where are essential for normal heart development. Calmodulin (CaM) has been implicated in mediating the Ca2+-dependent regulation of gap junctions. We have reported CaM binding site in the second half of intracellular loop of Cx43. In this study, two additional CaM binding regions in cytosol loop and C-termini of Cx43 have been identified by biophysical studies. Our results indicate that in the presence of Ca2+, synthesized Cx peptide fragments encompassing predicted CaM binding regions are able to bind with high affinity to CaM using NMR spectroscopy. Our results elucidate the molecular level of regulation of Cx43 by multiple CaM targeting regions and provide insight into molecular basis of gap junction gating mechanism.

544-Pos Board B330

Regional Connexin43 De-Phosphorylation and AMP-Kinase Activation after Rapid Pacing in Myofilament Ca Sensitized Hearts

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Increased myofilament Ca^{2+} sensitivity, a common finding in hypertrophic cardiomyopathy (HCM), is associated with an increased susceptibility for arrhythmias. In hearts expressing a Ca sensitizing troponin T (I79N) HCM mutation reentry-type arrhythmias are induced by fast pacing of isolated perfused hearts. Fast pacing also triggers arrhythmias in control hearts pre-treated with the Ca^{2+} sensitizer EMD57033. Regional conduction velocity (CV) slowing may contribute to formation of the arrhythmogenic substrate, but the underlying mechanism has not been elucidated.

Hypothesis: We hypothesized that a change in intercellular coupling via gap junctions (GJ) is responsible.

Methods: We analyzed connexin 43 (Cx43) by Western blot in total and soluble heart tissue fractions (three phosphoisoforms could be separated: P0,P1,P2), and via confocal imaging of immunostained frozen heart sections.

Results: Under baseline conditions, no difference was detectable in Cx43 expression level, soluble/insoluble ratio and isoform distribution in all assays (all $n \geq 7$). However, after hearts were rapidly paced (12-18 Hz for 16 min), Cx43 expression was lower in I79N compared to control ($64.8 \pm 6.6\%$, $n \geq 7$, $p = 0.03$) and the P0/P2 ratio increased ($p = 0.01$). Strikingly, immunostaining revealed areas with high accumulation of Cx43-P0 isoform (clusters) in I79N as well as EMD-treated hearts, while there was low homogeneous Cx43-P0 staining in controls. GJs are metabolically sensitive, so we next investigated AMP-kinase activation via phosphorylation at T172. Phosphorylation level was increased after pacing in I79N (to $225 \pm 47\%$ of control). The activated kinase accumulated in the same regions where Cx43-P0 staining was observed.

Conclusion: We conclude that during rapid pacing some regions become energy depleted, as evidenced by AMP-kinase activation. This triggers Cx43-P0 accumulation in the same regions and may contribute to the regional slowing of CV and thus formation of the arrhythmogenic substrate in Ca sensitized hearts.

545-Pos Board B331

Genetically De-Sensitizing Myofilaments to Ca in a Mouse Model for Hypertrophic Cardiomyopathy Decreases Arrhythmia Incidence

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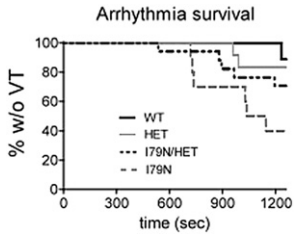
Increased myofilament Ca sensitivity, a common finding in hypertrophic cardiomyopathy (HCM), is associated with an increased susceptibility for arrhythmias. In isolated hearts expressing a Ca sensitizing troponin T (I79N) HCM mutation fast pacing induces reentry-type tachycardia (VT) in 60% of the hearts. We hypothesized that reducing myofilament Ca sensitivity will decrease arrhythmia incidence.
Methods: We generated knock-in mice with a TnT-R141W (HET) mutation, which is associated with dilated cardiomyopathy and reduced myofilament Ca sensitivity, and crossed them with I79N mice (CROSS). We determined heart weight-to-body weight ratio (HW/BW), cell diameter and fibrosis content and we monitored arrhythmia incidence in isolated hearts.
Results: The HW/BW ratio was 6.6 ± 1.1 in the WT, with 5.6 ± 0.9 lower in the I79N, with 7.6 ± 1.0 higher in the HET and similar to I79N in the CROSS (5.8 ± 1.0). In the CROSS cell diameter was smaller than in the HET and fibrosis content attenuated compared to I79N. Importantly, VT incidence in the CROSS was similar to control (23.5% vs 11.1% in WT).
Conclusions: Correcting myofilament Ca sensitivity reduces arrhythmia incidence of I79N mice and attenuates pathological remodeling observed in mice expressing each mutation alone.



546-Pos Board B332

The Dynamic Clamp Induced Pacing in Single and Coupled Cells

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The dynamic clamp is a method whereby an arbitrary current, a function of time and membrane potential, is applied to a preparation to create hybrid circuits of real and virtual cells.
We tested the ability to add electronically two membrane currents, the inward rectifier current (IK1) and the pacemaker current (If), which contribute to myocyte pacemaker activity. Injection of computer-simulated If in addition to IK1 under current-clamp conditions into an isolated canine ventricle myocyte and/or HEK293 cell stably expressing SCN5a initiated pacing activity. The pacing rate and action-potential (AP) duration were modified by changing If and IK1 amplitudes.
We also examined whether coupling of a source cell with If and IK1 to another cell creates a two-cell pacing unit. HEK293 cells were co-cultured either with HEK293/SCN5a cells or acutely isolated adult canine ventricular myocytes. Injecting If and IK1 currents into one cell of a pair generated sustained pacing in a coupled ventricular myocyte or HEK293/SCN5a cell once a critical level of electrical cell-cell coupling between the If+IK1 source cell and the neighboring cell was

exceeded. Application of the gap-junction blocker carbenoxolone stopped pacing activity in the coupled myocyte and/or HEK293/SCN5a cell; washout of carbenoxolone restored activity. Hence, the currents from a If+IK1-injected cell delivered to a cardiac myocyte (or another cell type) via gap junctions can generate spontaneous APs allowing the cell pair to function as a pacemaker unit.
Pacing activity also was investigated artificially by electrically connecting two separate single cells via a dual-cell dynamic clamp, permitting varying of coupling conductance while modulating If and/or IK1 within either or both of the two coupled cells. These results demonstrate that the dynamic clamp can be used to study the determinants of pacemaker activity.
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547-Pos Board B333
Gap Junction-Mediated Network Activity Promote Neural Progenitor Proliferation
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Cell Membrane Physiology & Biophysics
4A Intercellular Communication & Gap Junctions
The intercellular communication during early embryonic development is poorly understood. Here we report that mouse neural progenitor cells are forming functional networks that exhibit correlated spontaneous calcium (Ca^{2+}) activity that stimulate cell proliferation. By using mathematical cross-correlation analyses of single cell real-time Ca^{2+} recordings and network theory, we reveal highly synchronous activity in small world networks that follow a scale-free topology. These network formations of progenitor cells are interconnected by functional gap junctions that can transmit electrical current to trigger repetitive Ca^{2+} influxes. Pharmacological or molecular inhibition of network Ca^{2+} activities suppresses progenitor proliferation *in vitro* and *in vivo*. Taken together, these results demonstrate a novel function for early intercellular signaling networks that stimulate proliferation of neural progenitor cells.

548-Pos Board B334
A Chemical Chaperone Attenuates Pressure-Overload Induced Cardiac Hypertrophy by Reducing ER Stress
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Previously, it was reported that the endoplasmic reticulum (ER) stress could be involved in the pathological hypertrophy and the antagonism of angiotensin II type receptors could prevent ER chaperone expression and apoptosis (Kenichiro Okada *et al*. *Circulation*. 2004). We investigated whether the direct alleviation of ER stress by treating with a chemical chaperone could attenuate the hypertrophy. Transverse aortic constriction (TAC) was applied to induce pressure-overload hypertrophy in mice. The chemical chaperone was orally administered in the TAC operated mice (100mg/kg, daily). One week later, the heart was harvested for molecular study. The chemical chaperone/TAC group showed a reduction of ER stress, attenuation of hypertrophy and elimination of fibrosis compared with water/TAC group. We found down-regulation of GRP78/94, 10% attenuation of HW/BW ratio as well as the down-regulation of the hypertrophic marker genes compared with water/TAC group. The expression of collagen 1α1, 1α2, 3α1, TGF-β1, β3 and TGF-βR2, and the phospho-Smad2 responsible for the fibrosis were completely down-regulated in the chemical chaperone/TAC group compared with water/TAC group. Our results suggest that overload of protein in ER raises the unfolded protein response (UPR) in TAC model. The UPR could activate the MAP kinase which participated in the TGF-β up-regulation. In turn, TGF-β could aggravate the fibrosis by SMAD pathway. But the chemical chaperone helps to relieve the ER stress in pathological hypertrophy. As a result, the hypertrophy and the fibrosis could be reduced by down-regulation of MAPK - TGF-β - SMAD pathway. (Supported by Korea MEST NRF Grant (20110002144), the 2011 GIST System Biology Infrastructure Establishment Grant and KISTI-KREONET).

549-Pos Board B335
Non-Cholinergic Acute Sympathetic Stress Enhances Electrical Coupling in the Mouse Adrenal Medulla
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Sympathetic nervous system activity elicits catecholamine release from adrenal medullary chromaffin cells through cholinergic synaptic input from the innervating splanchnic nerve. Under basal sympathetic tone, the cholinergic system tightly regulates catecholamine release by requiring discrete acetylcholine-triggered action potentials for chromaffin cell catecholamine release. On the tissue-level, the cholinergic system exerts tonic inhibition of cell-cell electrical coupling. Under sustained splanchnic firing, as under the sympatho-adrenal stress response, chromaffin cells undergo desensitization to further cholinergic excitation. Yet, robust adrenal catecholamine secretion persists. This has been shown to be in response to a non-cholinergic peptidergic transmitter, PACAP. Pituitary Adenylate Cyclase Activating Peptide (PACAP) is an excitatory peptide transmitter released by the splanchnic nerve specifically under elevated firing. PACAP stimulates sustained catecholamine release through a PKA/PKC-dependent pathway that is mechanistically independent of the cholinergic pathway. In this study, we test the hypothesis that PACAP excitation is at least in part augmented by counteracting the cholinergic-suppression of gap junction coupling and functionally increasing cell-cell electrical coupling. We utilize perforated patch electrophysiological recordings conducted in adrenal tissue slices to investigate this hypothesis. We report that PACAP, via PKA/PKC signaling, increases degree of electrical coupling in mouse adrenal chromaffin cells by >40%. Thus, PACAP acts not only as a secretagogue, but it is also capable remodeling the adrenal medulla, presumably to adapt to the organism's needs during acute sympathetic stress.

550-Pos Board B336
The Endoplasmic Reticulum, A Novel Organelle for PKCδ Translocation
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Protein Kinase C delta (PKCδ), a member of the novel PKC family, is ubiquitously expressed and involved in many intracellular signal pathways. Compared to conventional PKCs, the translocation behavior of the nPKCs is unclear, although they also contain C1 and C2 domains. We found that, when co-expressing fluorescent protein fusion proteins of PKCα and PKCδ in the same cell, following ''physiological'' stimulation of the cells with ATP (100μM), PKCα translocated to the plasma membrane and PKCδ to intracellular membrane structures that we identified as the ER. In order to investigate whether C1 or C2 domains of the PKC were responsible for such a different redistribution patterns, we designed several chimera PKCs by exchanging C1 and C2 domains between PKCα and PKCδ and analysing subcellular translocation with various stimuli. Chimera II and III with their α-C2 domain not only displayed rapid and Ca^{2+} dependent plasma membrane translocation but also retained the slower ER translocation of wt-PKCδ. In contrast, chimeras I and IV did not display ATP or Ca^{2+} dependent translocation while their PMA-induced behavior was still intact. These data demonstrated the independent translocation capabilities of C1 and C2 domains in PKCs. But we were still puzzled to why the C1 domains of PKCs cause different recruitment schemes. To further enlighten this, we designed constructs in which we swapped C1a and C1b domains of PKCα and PKCδ. Expression and translocation experiments unequivocally showed that the C1b rather than C1a domain of PKCδ determined ER targeting, which was further confirmed by single amino acid mutation in C1b. To elucidate the signalling cascade contributing to PKCδ translocation, several specific chemical compounds and genetic inhibitors were applied. The results strongly indicated that the Gαs-cAMP-EPAC signal pathway played an essential role in the ER targeting PKCδ translocation.

551-Pos Board B337
A New Methodology for Quantitative LSPR Biosensing
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Localized surface plasmon resonance (LSPR) biosensing holds the promise of being able to map out the spatio-temporal nature of protein secretions of individual cells or controlled drug delivery devices in a label-free manner. Before these applications can be realized, however, it first must be shown that LSPR can be used for detecting protein concentrations on length and time scales that are applicable to such mass-transport limited processes, roughly 10 μm and 1 second, respectively. In this work a quantitative analysis methodology for LSPR biosensing is presented by which surface-receptor fractional occupancy as well as the local analyte concentration can be determined to within these specifications. Gold nanostructures of varying size were patterned into 20 x 20 arrays atop a glass coverslip using electron-beam nanolithography. The pitch between nanostructures was such that each array had a dimension of 10 μm x 10 μm or less. Association kinetics were measured on an inverted microscope, using Koehler illumination and cross-polarization microscopy for enhanced signal-to-background. An analytical model relating resonance peak perturbations to analyte binding at the surface was developed to determine the time-dependence of the surface-receptor fractional occupancy, $f(t)$. Once found, $f(t)$ was used in conjunction with the second-order reaction-diffusion equations to calculate the analyte concentration, $c(t)$. With this methodology, an individual biotinylated array was used to determine $f(t)$ and $c(t)$ of neutravidin analyte as well as that of antibiotin analyte in concentrations ranging from 1 μM to 50 nM. By regenerating the same array, a reliable comparison of the kinetics could be made between control sample (non-specific) kinetics versus that due to specific binding. The optical configuration is also consistent with fluorescence and DIC microscopy which can be used to augment the LSPR measurements.