used to guide or parameterize our modeling, and also can be used to compare with our simulation results. Current simulation results show that different adhesion energetic parameters can lead to different multi-cellular patterns, consistent with the experimental data. Future improvements and potential applications are also discussed.

1449-Pos Board B293

Relating Electrical Conductance, Connexin 43 Immunostaining, and Cell Shape in Micropatterned Cardiac Cell Pairs

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Gap junctions are responsible for cell-cell electrical coupling and maintaining normal cardiac conduction patterns. Connexin 43 (Cx43) is the dominant gap junction protein in ventricular myocytes. Although the relationship between Cx43, conduction, and tissue structure have been extensively studied in engineered monolayers of cardiac tissue, there have been few studies comparing conductance, Cx43, and cell shape on the cell-cell level. We have used micropatterning to control the dimensions of myocyte pairs and study electrophysiological properties at very high resolution. We hypothesized that conductance and Cx43 immunostaining would be directly correlated. We also sought to relate our electrophysiological measurements to cell shape. Using a dual voltage clamp system, we measured the conductance of micropatterned ventricular myocyte pairs, and subsequently fixed and immunostained the same cells for Cx43. Thus, we compared conductance and Cx43 immunostaining serially in the same cell pair. The volume of Cx43 immunostaining was determined using confocal microscopy and quantitative software programs. Using brightfield images, we measured cell dimensions and 2-dimensional cell-cell contact. We studied three types of rectangular cell pairs with varying length to width aspect ratios (3.33:1, 5:1, 6.67:1). The average length of the cell-cell junction ($R2=0.99$, $n=23$), average conductance ($R2=0.92$, $n=22$), and average Cx43 immunostaining ($R2=0.85$, $n=22$) increased linearly relative to the aspect ratio. We found a linear relationship between Cx43 immunostaining and conductance ($R2=0.70$, $n=22$). A weaker linear relationship was found between conductance and 2-dimensional length of the cell-cell junction ($R2=0.51$, $n=23$). Our results suggest that cell pairs nearly maximize their contact area, which contributes to increases in both Cx43 and conductance. Cell pairs with higher length-width aspect ratios have more cell-cell contact and therefore higher Cx43 density and conductance. However, Cx43 density is the most important determinant of conductance.

1450-Pos Board B294

Role of connexin 32 hemichannels in ATP release from Schwann cells

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The X-linked Charcot Marie Tooth (CMTX) is an inherited disease due to mutations in connexin 32 (Cx32) gene expressed in Schwann cells (SC) of peripheral nerves. In SC, Cx32 localizes in the paranodes, in the Schmidt Lanterman incisures and in the cell surface. Cx32 can form "reflexive" gap junction channels as well as functional hemichannels open upon membrane depolarization. We have explored the permeability of Cx32 hemichannels to ATP, in SC and in a heterologous expression system. Murine sciatic nerve trunks release ATP under electrical or mechanical stimulation, as determined by the luciferase reaction. Luminescence imaging revealed that ATP release is especially intense at the SC paranodes, which contain the highest immunofluorescent label for Cx32. Cultured adult SC have a high expression of Cx32 and under mechanical stimulus release ATP being insensitive to exocytosis blockers like brefeldin A. In *Xenopus* oocytes expressing human Cx32, we measured simultaneously the hemichannel currents and the release of ATP elicited by a square depolarizing pulse up to +100 mV. Depolarizing pulses induced characteristic slowly activating outward currents and when the membrane potential returned to the holding voltage tail currents coinciding with the peak of ATP release. The deconvolution of the light signal revealed that the time courses of the tail current and the ATP release were coincident. We established a direct relationship between the amount of ATP released and the amplitude of tail current. Applying positive voltages closer to the ATP reversal potential during the tail current reduced the amount of ATP released. Five different single amino acid mutants of Cx32, described in CMTX, affecting intracellular, extracellular or transmembrane domains, were tested. Those mutations deeply inhibited or abolished the hemichannel currents and the ATP release.

1451-Pos Board B295

Regulation Of Neuronal Connexin-36 Channels by pH

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Neurotransmission through electrical synapses plays an important role in the spike synchrony among neurons and oscillation of neuronal networks. Indeed, electrical transmission has been implicated in the hypersynchronous electrical activity of epilepsy. We have investigated the influence of intracellular pH (pHi) on the strength of electrical coupling mediated by connexin36 (Cx36), the principal gap junction protein in the electrical synapses of vertebrates. In striking contrast to other connexin isoforms, the activity of Cx36 channels decreases following alkalosis rather than acidosis when it is expressed in Xenopus oocytes and N2A cells. This uncoupling of Cx36 channels upon alkalinization occurred in the vertebrate orthologues analyzed (human, mouse, chicken, perch and skate). While intracellular acidification caused a mild or moderate increase in the junctional conductance of virtually all these channels, the coupling of the skate Cx35 channel was partially blocked by acidosis. The mutational analysis suggests that the Cx36 channels may contain two gating mechanisms operating with opposing sensitivity to pH. One gate, the dominant mechanism, closes for alkalosis and it probably involves an interaction between the C- and N-terminal domains, while a secondary acid sensing gate only causes minor, albeit saturating, changes in coupling following acidosis and alkalosis. Thus, we conclude that neuronal Cx36 channels undergo unique regulation by pHi since their activity is inhibited by alkalosis rather than acidosis. These data provide a novel basis to define the relevance and consequences of the pH-dependent modulation of Cx36 synapses under physiological and pathological conditions.

1452-Pos Board B296

Propagation of Fast and Slow Intercellular Calcium Waves in Primary Cultured Smooth Muscle Cells

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Tissue blood flow is controlled by the changes in the diameter of the arteries and arterioles due to the coordinated contraction and relaxation of smooth muscle cells (SMCs) within the vascular wall. The contractile state of SMCs is regulated primarily by the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ in response to hormonal stimuli propagates from cell to cell along the vessel wall as a wave, and activates the process of contraction. The mechanism underlying this phenomenon, however, is not yet fully revealed.

In this work, we study the onset and propagation of intercellular calcium waves through gap junctions in primary cultured vascular SMCs. For imaging intercellular Ca^{2+} waves, SMCs seeded along a collagen line and loaded with the fluorescent Ca^{2+} indicator Fluo-4 were locally stimulated mechanically or chemically. The stimulation evoked two distinct calcium waves: 1) a fast Ca^{2+} wave (several mm/s), and 2) a much slower Ca^{2+} wave (few tens of μm/s); both waves propagated to neighboring cells. The fast Ca^{2+} wave was caused by the propagation of membrane depolarization and subsequent Ca^{2+} influx through voltage operated channels. This fast wave facilitated the onset and propagation of a slow, but higher amplitude Ca^{2+} wave that started from the stimulated cell and propagated to neighboring cells. Our results suggest a possible mechanism for intercellular Ca^{2+} wave propagation through gap junction channels in SMCs.

1453-Pos Board B297

Connexin Pore Block By ABG-Sugars

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Pore blockers are valuable for structure-function study of membrane channels. Prior work shows novel anthranilamide moieties (ABG) derivatized to maltosaccharides of different lengths (Gn: n-glucose) are size-indexed pore blockers of connexin channels: block occurs with size-match with a segment of the pore lumen, not if the lumen or blocker is too narrow or wide. Permeation studies using the same maltosaccharides derivatized with an uncharged fluorescent group (PA-sugars) show the narrowest part of the pore (size-selective filter) decreases $Cx32>Cx26\approx Cx26/Cx32$, the last being heteromeric. Efficacy studies of ABG-sugar block from each side of the pore reveal new information about

channel structure: connexin pores have constricted segments with which ABG-sugars interact, other than the size-selective filter. From the cytoplasmic side, ABG-G3 blocks Cx26 but not Cx32 or Cx26/Cx32 channels, and the wider ABG-G4 blocks with decreasing effectiveness Cx32>Cx26>Cx26/Cx32. From the extracellular side, ABG-G3 blocks Cx26/Cx32 better than Cx26, and does not block Cx32 channels, while ABG-G4 has no effect on any channels tested. If block were exclusively at the size-selective filter, the pattern of block from both sides of the pore should be identical and consistent with the PA-sugar pore sizing study. Instead, the data show that pore width varies as the selectivity filter is approached from one side or the other. Specifically, the pore lumen of homomeric Cx26 and Cx32 channels narrows on the cytoplasmic side of the selectivity filter. Intriguingly, heteromeric Cx26/Cx32 channels show unique and substantial narrowing extracellular to the selectivity filter. This is significant as most connexin channels in vivo are heteromeric, and heteromeric channels are selective amongst second messengers while the corresponding homomeric channels are not. Our data suggest a consequence of 'heteromericity' is that segment/s of a pore-lining domain are asymmetrically displaced toward the center of the lumen. Supported by GM36044, NS56509.

1454-Pos Board B298

Chemical Gating Mechanism Of Connexin26-containing Channels By Aminosulfonate

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Protonated taurine directly and reversibly inhibits homomeric and heteromeric Cx26-containing hemichannels but not homomeric Cx32 hemichannels. It is unknown if taurine interacts with Cx26 and/or Cx32 in heteromeric channels, which domains are involved, or if junctional channels are taurine-sensitive. These issues were addressed with channels composed of Cx26 and/or Cx32 with/without cleavable 3KDa carboxyl-terminal (CT) tags (T). Hemichannel activity was assessed in liposomes, and by extracellular dye-uptake in cells. In contrast to untagged hemichannels, Cx26T/Cx32 and Cx26T hemichannels were not taurine-sensitive, but Cx26/Cx32T hemichannels were. Tag cleavage (Tc, leaving 4aa at the carboxyl-terminus) restored taurine-sensitivity of Cx26Tc/Cx32 hemichannels, but taurine surprisingly narrowed rather than closed Cx26Tc hemichannels. Thus, the 3KDa CT tag blocks taurine-sensitivity, unless hemichannels also contain Cx32, and the short 4aa CT extension affects Cx26Tc channel open state. Taurine effects on junctional channels were assessed by intercellular dye-coupling. Taurine substantially reduced dye-coupling by Cx26 and Cx26/Cx32T channels, but not by Cx26T, Cx26T/Cx32 or Cx32T channels. Junctional channels therefore have identical taurine-sensitivity as their component hemichannels. An intracellular site for taurine action was shown by a membrane-impermeable blocker of taurine uptake. Thus, all data indicate taurine-induced pore closure utilizes the Cx26 CT. Taurine binding to Cx26-CT was assessed by natural-abundance 13C-HMQC-NMR. Overlapping resonances of Cx26-CT peptide in the presence and absence of taurine indicate no direct taurine binding to Cx26-CT. Peptide 'elisa' showed a pH dependent interaction occurs between Cx26-CT and the carboxyl-terminal 20aa of the Cx26 cytoplasmic loop (Cx26-CL). Acidification increases the binding affinity of Cx26-CL and Cx26-CT peptides, and only the protonated form of taurine negatively affects this interaction, suggesting that its disruption leads to channel closure. Structural analysis of Cx26-CT and Cx26-CL peptides in the presence and absence of taurine are ongoing. Supported by GM36044, DC7470.

1455-Pos Board B299

Quantitative Experimental Measurements And Mathematical Modeling Of Multi-cellular Dynamics In The Islet Of Langerhans

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Multi-cellular interactions and dynamics are key to mammalian physiology. Many organs such as the heart or brain have complex functionality that is brought about by the interactions between constituent cells. To discover general rules and principles that govern multi-cellular behavior, we are studying a relatively simple mammalian multi-cellular system- the islet of Langerhans. The islet is located in the pancreas and provides the sole source of the hormone insulin for regulating blood glucose levels. Thus, understanding the function of the islet is of critical importance to effectively treat diabetes. In this work, we have focused on understanding the electrical dynamics in the islet that underlie the coordinated pulsatile secretion of insulin. We have used quantitative microscopy to measure intra-cellular free calcium activity ($[Ca^{2+}]_i$) simultaneously over a large cellular population of the islet. To provide an experimental axis, we introduced graded changes in the electrical coupling between beta cells by applying both chemical inhibitors of gap junction activity as well as a genetic knockout of the gap junction protein. Upon this reduction in electrical coupling, synchronization of pulsatile electrical activity decreased throughout the islet. Furthermore, the propagating $[Ca^{2+}]_i$ waves, which serve to synchronize electrical oscillations, slowed and were disrupted as electrical coupling was reduced. Using a mathematical model of islet cell electrical activity and multi-cellular coupling, we can quantitatively reproduce this experimental data. This allows us to make quantitative predictions of the multi-cellular electrical behavior for any given level of electrical coupling, as well as expected behaviors under perturbations of other parameters, such as K^+-channel mutations. We can also hypothesize that this behavior is general for electrical coupling in other multi-cellular systems, potentially allowing us to predict the electrical dynamics in other neuro-endocrine cell systems.

1456-Pos Board B300

Triarylmethanes - a New Class of Connexin Blockers

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Connexins (Cx) are a family of proteins with 4 transmembrane regions, which are encoded by 21 genes in humans and which form hexameric connexons (= hemichannels). These connexons can either function as transmembrane ion channels or assemble into gap junctions that directly mediate signaling between adjacent cells by allowing the passage of ions, metabolites and signaling molecules up to 1 kd in mass. Both gap junctions and hemichannels play important roles in many tissues and have therefore been proposed as potential new targets for the treatment of epilepsy, cardiac arrhythmia and cancer. However, there are no specific and potent pharmacological tools to study the physiological as well as the pathophysiological role of connexins. The existing connexin modulators are either of low potency or cross-react with other ion channels. In order to identify potent and selective connexin blockers we screened a small library of compounds containing pharmacophores known to modulate other ion channels. From this library, we identified four new small molecule chemotypes including triarlymethanes (TRAMs) like clotrimazole and benzimidazoles like astemizole that inhibit Cx50 channels in a sub-type specific manner with IC50 values in the range of 1-10 μM while having little or no effect on those formed by Cx46, Cx36 and Cx32. We are currently exploring the structure activity relationship (SAR) of TRAMs for Cx50 inhibition and have recently identified T66 (N-[(2-chlorophenyl)(diphenyl)-methyl]-N-(1,3-thiazol-2yl)amine), which exhibits an IC50 of 3 μM. In general, the SAR of the Cx50 inhibiting TRAMs significantly differs from the SAR of KCa3.1 blocking TRAMs. We propose T66 and its derivatives as novel pharmacological tool compounds that may be used to study the physiological role of connexins.

1457-Pos Board B301

Pro-arrhythmic Effects Of Fibroblast-myocyte Coupling In Simulated Cardiac Tissue

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Fibroblasts comprise the majority of non-cardiac cells in normal heart and mediate the structural remodeling underlying progressive fibrosis in cardiac diseases. Recent experimental studies have shown that fibroblasts can electronically couple to myocytes via gap junctions and alter myocyte electrophysiology. However, the implications for cardiac arrhythmias are incompletely understood. In this study, we used mathematical modeling and computer simulation to investigate how fibroblast-myocyte coupling affects the dynamics of action potential duration (APD), excitation-contraction coupling, and alternans. Our major findings are: 1) Fibroblast-myocyte coupling shortens APD when fibroblast membrane conductance is high and resting membrane potential is low, but prolongs APD for other choices of conductance and resting potential. 2) Depending on the membrane conductance and resting potential of fibroblasts, fibroblast-myocyte coupling can either promote or suppress APD alternans by steepening or flattening APD restitution. 3) When alternans is calcium-driven, fibroblast-myocyte coupling always promotes alternans, and can result in electromechanically discordant alternans. 4) In cardiac tissue, fibroblast-myocyte coupling slows conduction velocity and broadens its restitution, promoting