spatially discordant alternans. Spatially discordant alternans can also arise from electromechanically concordant alternans and electromechanically discordant alternans in different regions of the tissue. In conclusion, fibroblast-myocyte coupling has multiple pro-arrhythmic effects on electrophysiological properties in cardiac tissue.

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Gap Junction Permeability: Transfer of Negative and Positive Charged Probes

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Gap junction channels are composed of connexins, which exhibit specific permeability to the variety of larger solutes including second messengers, polypeptides and siRNAs.

Here, we report the permeability of solutes with different size and net charge Lucifer Yellow (443 (no counterion), -2); Carboxyfluorescein (376, -2); AlexaFluor350 (326, -1); Ethidium bromide (314, +1) and NBD-m-TMA (280, +1) through gap junction channels in HeLa cells expressing Cx26, Cx40, Cx43 and Cx45.

The channel permeability was determined using simultaneous measurements of junctional conductance and the cell-cell flux of a fluorescent probe.

All four connexins transferred negative charged probes: LY, CF and AF350, however, Cx40 and Cx26 exhibited reduced permeability when compared to Cx43. These connexins revealed following permeability ratios for LY/ CF/ AF350, respectively, relative to the ubiquitous cation K+: 0.029/ 0.032/ 0.069 for Cx43; 0.014/ 0.021/ 0.049 for Cx45; 0.0044/ 0.014/ 0.0245 for Cx26 and 0.0026/ 0.0034/ 0.0206 for Cx40.

The positive charged NBD and EthBr exhibited the following permeability relative to K+: 0.045 and 0.0125 for Cx43; 0.048 and 0.0036 for Cx45; 0.055 and 0.026 for Cx26; 0.040 and 0.009 for Cx40.

In summary, all negative charged species showed a similar permeability order: Cx43>Cx45>Cx26>Cx40. For positively charged species the permeability orders were: $Cx26 \approx Cx43 \approx Cx40 \approx Cx45$ (NBD) and $Cx26 \geq Cx43 \approx Cx40 > Cx45$ (EthBr). Reduced EthBr permeation through Cx45 channels in comparison to other connexins suggests a size-dependent discrimination of the solute. However, the reduced correlation between junctional conductance and positive charged probes flux suggests intracellular binding of the solute. Therefore quantitative comparison of positively charged solutes has to be taken cautiously.

These results confirm that channels formed from individual connexins can discriminate for solutes based on size and charge suggesting that channel selectivity may be a key factor in cell signaling.

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Stem Cell Transplantation Induces a Rapid Change in Cardiac Excitability

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Embryonic (ES) and bone marrow derived stem cells are discussed as a potential source for cardiac replacement tissue. Transplantation of undifferentiated cells into the cardiac infarct region has been shown to decrease infarct size and preserve cardiac function. Long term studies determined homing of stem cells in the cardiac muscle, the immediate impact of cell transplantation on the electrophysiological properties of however remains unclear. To determine if the time course in which stem cells establish intercellular coupling we plated Calcein/AM loaded ES cells on monolayers of cardiomyocytes (HL-1 cells). Dye transfer that was monitored by confocal microscopy, was first observed 60 min after heterocellular culture was established. It could be blocked by carbenoxolone indicating the presence of gap junction channels. After 200 min 36 ± 7% of ES cells had established intercellular coupling with cardiomyocytes. To determine the impact of cell transplantation on the electrophysiological properties we established monolayers on multielectrode arrays (MEAs). From field potential measurements we determined that induction of co-culture resulted in a biphasic change of the electrophysiological properties. During the first 45 min an increase of the conduction velocity (CV: 142 ± 17 %) and of the spontaneous beating frequency (F: 172 ± 11%) could be detected (n = 10). With further progression of co-culture however, a continuous decrease of excitability occurred (180min; F: 31 ± 3%; CV: 50 ± 2.5%) that ultimately resulted in the loss of spontaneous activity (210 min). In control cultures no biphasic change in F or CV was observed. The data indicate that stem cell transplantation results in rapid heterocellular coupling between stem cells and cardiomyocyte and a suppression of cardiac excitability. The contribution of intercellular coupling and other paracrine mechanism to the change in excitability remains to be determined.

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Posttranslational Modifications Of Connexin26 Identified By MALDI-TOF/TOF Mass Spectrometry

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Gap junctions play important roles in auditory function. Mutations in the Cx26 gene are the predominant cause of inherited nonsyndromic deafness. Some Cx26 deafness mutations directly disrupt the intercellular molecular and/or ionic signaling pathway, while others may affect the location and character of posttranslational modifications (PTMs) governing channel assembly, function and biological regulation. Mass spectrometry was used to determine if Cx26 PTMs occur at sites of deafness-causing mutations. Cx26 was isolated from HeLa cells at >95% purity by immunoaffinity followed by metal chelate chromatography using carboxyl-terminal hexahistidine and hemagglutinin tags. In-gel and in-solution enzymatic digestions were carried out in parallel with trypsin, chymotrypsin and endoproteinase-GluC. Peptides were recovered with and fractionated from reverse-phased C8 beads by stepwise elution with increasing concentrations of organic solvent. Using an ABI4800 MALDI-TOF/TOF-MS, spectra were acquired from each elution step, thereby improving detection of low abundance peptides in complex mixtures and maximizing sequence coverage. Acquisition, processing and interpretation parameters were further optimized to improve ionization and fragmentation of hydrophobic connexin peptides. MALDI-TOF-MS and MALDI-TOF-MS/MS sequence coverage values obtained were significantly above those reported for other mammalian membrane proteins. Total Cx26 sequence coverage by MALDI-TOF-MS was 75.2%, with 31.1% sequence confirmed by MALDI-TOF-MS/MS. Improved ionization and sequencing of Cx26 peptides (especially transmembrane pore-lining domains) were further achieved with a Waters nano-liquid chromatography-coupled electrospray ionization quadrupole-TOF-MS. Several different PTMs of Cx26 were identified, many of which were at sites of deafness-causing mutation. The PTMs included phosphorylation, acetylation, methylation, citrullination, hydroxylation, γ-carboxylation and palmitoylation. Knowledge of the location and character of Cx26 PTMs will be instrumental in guiding experiments to understand how cellular mechanisms of channel regulation can become altered and lead to losses in auditory function. Supported by GM36044, DC7470, NS56509 (ALH) & NS046593 (HL).

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Mutagenesis Of Charged Residues In The N-terminal α-helix Of Connexin37 Reveals An Essential Lysine Residue

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Connexins are transmembrane protein subunits that combine to form cell surface hexameric hemichannels and intercellular gap junction channels. They contain a short cytoplasmic N-terminus that has been implicated in channel gating and oligomerization. Using NMR, we previously showed that the N-terminus of human connexin37 (CX37) is α-helical between amino acids 5 and 16. The α-helical region has a hydrophilic face with three aligned negatively charged residues (E8, D12 and E16) and one positively charged residue (K9). To test their function, these charged amino acid residues were mutated individually to alanines or to other neutral amino acids. The CX37 mutants were tested for formation of gap junction plaques by fluorescence microscopy after transient transfection of HeLa cells and for formation of functional hemichannels by two-microelectrode voltage clamp after expression in single *Xenopus* oocytes. Each of the negatively charged amino acid alanine substitution (E8A, D12A, or E16A) or charge neutralization (E8Q, D12N, or E16Q) mutants formed gap junction plaques. These mutants all formed conducting hemichannels; ionic currents were of comparable magnitude to those in oocytes expressing wild-type CX37 when measured without divalent cations and were blocked by 2 mM external calcium. While gap junction plaques were observed in HeLa cells transfected with K9A, this construct did not form conducting hemichannels in *Xenopus* oocytes. No plaques were detected in a charge reversal mutant at this position (K9E). These results suggest that the negatively charged residues within the N-terminal α-helix are not individually required for formation of functional channels. In contrast, the positively charged residue, K9, is required for hemichannel opening and influences formation of gap junction plaques.

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Extracellular ATP Mediates The Intercellular Ca2+ Wave Induced By Mechanical Stimulation In Human Salivary Gland Cells

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