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.

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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.

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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.

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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.

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

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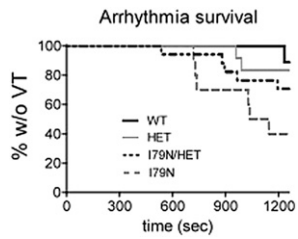
Increased myofilament Ca sensitivity, a common finding in hypertrophic cardiomyopathy (HCM), is associated with an increased susceptibility for arrhythmias. In isolated hearts expressing a Ca sensitizing troponin T (I79N) HCM mutation fast pacing induces reentry-type tachycardia (VT) in 60% of the hearts. 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.



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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