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FRET Measurements of cAMP Dynamics in HL1 Cells Support the Key Role of Constitutive AC Activity in Cardiac Pacemaking

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Spontaneously beating HL1 cardiac cells have evolved as a very useful tool to study regulation of pacemaking cardiac activity because of the ability to knock down specific molecules without generating KO mice in vivo. HL1 cells, however, have not been fully characterized and their pacemaker mechanisms may differ from those in cardiac pacemaking cells derived from adult heart. Basal constitutive adenylate cyclase activity is an obligate factor for normal spontaneous firing in adult sinoatrial nodal pacemaker cells.We expressed FRET sensors in spontaneously beating HL1 cardiac cells to measure cAMP concentration. The PDE inhibitor IBMX (100 μM) increased the basal concentration of cAMP more than twofold and increased beating frequency by 70%. AC1 knockdown with siRNA completely inhibited the IBMX-induced increase in cAMP production in cytoplasm and plasma membrane, and decreased the beating frequency of HL1 cells. Pharmacological inhibition of both plasma membrane AC (2′-5’ dideoxyadenosine) and cytoplasmic AC (KH7) selective blockers completely abolished the IBMX induced increase of cAMP concentration and resulted in complete cessation of spontaneous beating in HL1cardiac cells. Thus, as in adult sinoatrial nodal pacemaker cells, basal AC activity is required for the spontaneous beating of HL1 cardiac cells.

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Abnormalities in Transmural Ventricular Electrophysiology in a Heterozygous SCN5A Knockout Mouse Model Revealed by Two-Photon Microscopy

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The voltage-gated cardiac sodium channel Nav1.5, is a critical regulator of cardiac electrical excitability, responsible for the rapid upstroke of the action potential (AP) in both atrial and ventricular cardiomyocytes. In addition to regulating single cell electrophysiology Nav1.5 plays a critical role in conjunction with connexin43, in ventricular conductance. Mutations in the SCN5A gene encoding Nav1.5 are linked with several inherited arrhythmic disorders, including Brugada syndrome and long QT syndrome. Heterozygous mutant mouse models demonstrate severe conduction disturbances and intramural fibrosis, preferentially in the right ventricle (RV). Despite the observation that single cardiomyocyte upstroke velocity is significantly reduced, it has thus far been difficult to separate the relative contribution of reduced Nav1.5 conductance and intramural fibrosis to the appearance of lethal ventricular arrhythmias. We address this issue by characterizing in greater detail the transmural electrophysiological behavior of ventricular tissue in a mouse model of SCN5A downregulation. Heterozygous SCN5A+/− mutant mice (SCN5A+/−; n=7) and wild type (WT; n=6) littermates’ hearts were langendorff perfused beneath a combined 2P and epifluorescence microscope system and loaded with voltage (di-4 ANEPPS) and intracellular calcium (Fura-2AM) sensitive dyes. Under physiological endo-epicardial activation, no significant difference between groups was apparent from optical map recordings of the RV surface. Transmural 2P line scanning revealed significantly slower AP upstroke times within the subepicardium, but not close to the surface, in SCN5A+/− vs WT. Conduction velocity was slower in SCN5A+/− vs WT mice, and was preferentially slow in the midmyocardium vs epi/endocardium. Beat-to-beat activation was also significantly higher in SCN5A+/− vs WT hearts. These data suggest significant conduction abnormalities within the midmyocardial wall which affect the rate and path of conduction preferentially within this transmural region.

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Features of Optical Mapping in Blue-Green and NIR Lights in Rabbit Heart

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Optical signal is the image created from the propagating electrical signal. Due to optical features (absorption, scattering, etc.) of the heart tissue the coincidence between optical and electrical action potentials (APs) is moderate. Having electrical action potentials recorded transmurally, we aimed to evaluate contribution of the electrical and the optical features on OAP upstroke formation using blue-green and near-infrared (NIR) lights. Here we introduce a new approach in analysis of the electrical activity from the optical mapping (OM) studies by recording transmural-APs with glass-microelectrodes and comparing them with the OAP, obtained using blue-green (di-4-ANEPPS) and NIR (di-4-ANBDQBS) dyes in Langendorff-perfused rabbit heart under atrial/endo-/epicardial pacing. We used averaged upstroke of transmural-APs for isolation of electrical and optical (of dye/tissue) impacts influencing formation of OAP upstroke shape, and this had helped to split the OAP upstroke into components (depth-weighted and lateral-scattering). These components separately reflect the transmural and the parallel to the epicardium electrical propagating wave. In addition, to calculate depth-weighted component, we used the probing-depth constant for fluorescence measurement that was detected directly during the OM experiment, but not from separate measurements of the excitation and the emission light penetration. The detected probing-depth constant (k) for the NIR dye was ~2 mm, while that magnitude was about twice smaller for the blue-green dye. The results of the study open a new opportunity for the future investigations of the electrical impulse propagation in the heart.
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Comprehensive Analysis of Behavioral Variability in Real and Simulated Populations of Rabbit Left Ventricular Cardiomyocytes

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Variability in behavior between cells and individuals is an important yet poorly studied phenomenon. Studies of cardiac physiology generally focus on typical behavior rather than quantitatively examining variability, and the causes and implications of behavioral variability in these systems are largely unknown. One reason for this is that the low-throughput nature of most physiology experiments makes studies of variability a daunting challenge. We implement two complementary approaches to overcome this: population-based mathematical modeling and higher-throughput experimental methods.

We have applied population-based modeling approaches to two models of electrophysiology and calcium handling in the rabbit LV cardiomyocyte, creating a population of cells by varying parameters representing levels of ion channels and transporters. This analysis reveals several key features of behavioral variability in these populations, including: (i) the same population shows much greater variability in calcium transient (CaT) amplitude than in action potential duration (APD), indicating a potentially fundamental propensity for variability in CaTs, (ii) covariation occurs between outputs (e.g. positive correlations between CaT duration and APD) and enables prediction of the effects of some perturbations (e.g. partial block of L-type current) based on baseline behaviors.

This theoretical analysis is complemented with experimental measurements of variability using the recently-developed CellOPTIQ system, which allows repeat optical measurements of APs and CaTs to be made with increased throughput (up to 100 cells in 2-3 hours). Early measurements of APD50 from cells isolated from the LV of a single rabbit (n=85 cells) show an approximately normal distribution (mean=297 ms, SD=46 ms, range 151-445 ms), with cells from other rabbits showing similar distributions. These experimental measurements allow for comprehensive quantification of variability and testing of hypotheses generated by model analysis (e.g. correlations between outputs and different degrees of variability between outputs).

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Application of the RIMARC Algorithm to a Large Data Set of Action Potentials and Clinical Parameters for Risk Prediction of Atrial Fibrillation

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Ex-vivo recorded action potentials (APs) in human right atrial tissue from patients in sinus rhythm (SR) or atrial fibrillation (AF) display a characteristic

spike-and-dome or triangular shape, respectively, but variability is huge within each rhythm group. The aim of our study was to apply the machine learning algorithm RIMARC (Ranking Instances by Maximizing the Area under the ROC Curve) to a large data set of 480 APs combined with retrospectively collected general clinical parameters, and to test whether the rules learned by the RIMARC algorithm can be used for accurately classifying the pre-operative rhythm status. APs were included from 256 SR and 224 AF patients. During a learning phase, the RIMARC algorithm established a ranking order of features by predictive value for SR or AF. This was achieved by discretizing each continuous feature using a maximum area under ROC curve-based discretization (MAD2C) algorithm, and learning a ranking function for each feature, which is a linear combination of non-linear scoring functions learned. The model was then challenged with an additional test set of features from 28 patients in whom rhythm status was blinded. The accuracy of the risk prediction for AF by the model was very good (0.93) when all features were used. Without the 7 AP features accuracy still reached 0.71. In conclusion, we have shown that training the machine learning algorithm RIMARC with an experimental and clinical data set allows predicting a classification in a test data set with high accuracy. In a clinical setting this approach may prove useful for finding hypothesis-generating associations between different parameters.

Voltage-gated K Channels II

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Effect of Amitriptyline in Kv7.1/MinK Channel

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The KCNQ1 gene encodes the voltage-gated K^+ channel Kv7.1, which is mainly expressed in cardiac muscle. Coassembly with the β-subunit (MinK) Kv7.1 generates a very slowly activating delayed-rectifier K^+ current, I_{Ks}, with no apparent inactivation.

It has been reported that amitriptyline, a tricyclic antidepressant, inhibits the Kv1.1 and Kv7.2/7.3 K^+ channels in a voltage-independent but concentration-dependent manner. However, there is no evidence of the effect of this drug on Kv7.1; a channel of the same K^+ channel family than Kv7.2/Kv7.3 but with different kinetics and sequential characteristics.

Amitriptyline has been shown to induce long QT syndrome and torsades de pointes in human hearts which cause sudden death. This effect was related to HERG channel blockage, the molecular correlate of the rapid activated delayed rectifier K^+ current (I_{Kr}); however, the drug effects on I_{Ks}, a major determinant of action potential repolarization in the heart, has not been studied yet.

In this study we show that amitriptyline inhibits Kv7.1/MinK in a concentration-dependent manner with an IC_{50} of 3.27 μM. Inhibition of these channels was voltage-independent and reversible. The voltage dependence activation of the channel was not modified by amitriptyline. We assessed the effect of the drug on Kv7.1 channels assembled as homotetramer, without the accessory subunit. Kv7.1 channel current was less sensitive to inhibition by amitriptyline (IC_{50} 13.17 μM) than heteromeric Kv7.1/MinK channel current but like Kv7.1/MinK, current inhibition was voltage independent. Our results demonstrate that amitriptyline inhibits Kv7.1/MinK channels and we suggest that the drug acts on the pore forming subunit Kv7.1 instead of MinK.

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Polyunsaturated Fatty Acid Analogues Act Anti-Arrhythmic on the Cardiac IKs Channel

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Polyunsaturated fatty acids (PUFAs) affect cardiac excitability. Kv7.1 and the β-subunit KCNE1 form the cardiac IKs channel that is central for cardiac repolarization. In this study, we explore the prospects of PUFAs as IKs channel modulators. We report that PUFAs open Kv7.1 via an electrostatic mechanism. Charged n-3 and n-6 PUFAs affect the voltage dependence of Kv7.1 by shifting the conductance versus voltage curve towards more negative voltages. In contrast, uncharged methyl esters of the PUFAs do not affect the voltage dependence of Kv7.1. Both the polyunsaturated acyl tail and the negatively charged carboxyl head group are required for PUFAs to open Kv7.1. The PUFA effect is pH dependent. This is likely because high pH deprotonates the PUFA, making a larger fraction of PUFA molecules negatively charged and thereby able to affect Kv7.1 channel voltage dependence. We further show that KCNE1 co-expression abolishes the PUFA effect on Kv7.1 by promoting PUFA protonation. PUFA analogues with a decreased pKa value, to preserve their negative charge at neutral pH, restore the sensitivity to open IKs channels. PUFA analogues with a positively charged head group inhibit IKs channels. These different PUFA analogues could be developed into drugs to treat cardiac arrhythmias. In support of this possibility, we show that a PUFA analogue with a permanently negatively charged head group acts anti-arrhythmic in cardiomyocytes. This permanently negatively charged PUFA analogue induces a shortening of action potential duration in embryonic rat cardiomyocytes and restores rhythmic beating in an arrhythmia model.

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Intracellular Calcium Alters IKs Amplitude and Kinetics in Rabbit Myocytes

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The slowly activating delayed rectifier K^+ current (I_{Ks}) contributes to repolarization of the cardiac action potential (AP). Intracellular Ca^{2+} ($[Ca^{2+}]_i$) can modulate I_{Ks}, but details of this potentially important regulation are largely unknown. Here, we aimed at assessing $[Ca^{2+}]_i$ regulation of I_{Ks} and how it governs myocyte AP duration. I_{Ks} was recorded from freshly isolated rabbit ventricular myocytes using the patch clamp technique with intracellular pipette solutions that buffered $[Ca^{2+}]_i$ to 0, 100, 300, and 500 nM. From a holding potential of −50 mV, I_{Ks} was recorded by implementing step-like pulses from −40 to 50 mV in 10 mV increments for 3 s, followed by a tail-pulse to −50 mV for 3 s. When the $[Ca^{2+}]_i$ was increased to 300 nM, the maximally activated tail I_{Ks} (I_{MAX}) was more than 2-fold greater compared to 0 nM $[Ca^{2+}]_i$ (I_{MAX} ~0.8 pA/pF *vs.* 0.3 pA/pF). Importantly, when the pipette solution contained 500 nM $[Ca^{2+}]_i$ (I_{MAX} ~1.0 pA/pF), I_{MAX} was 3-fold greater than 0 nM and more than 2-fold greater than 100 nM $[Ca^{2+}]_i$ (I_{MAX} ~0.4 pA/pF). The potential of half-maximal activation (V½) was not different for any situations (~15 mV). However, deactivation kinetics of tail I_{Ks} were slower for cells recorded with 300 and 500 nM $[Ca^{2+}]_i$ compared to 0 and 100 nM $[Ca^{2+}]_i$ (τ_{deact} ~1200 ms *vs.* 800 ms). These results indicate that a rise in $[Ca^{2+}]_i$ increases I_{Ks} amplitude in rabbits, without altering the voltage dependence of activation, and slows I_{Ks} deactivation. Computational modeling suggests these $[Ca^{2+}]_i$-dependent changes might contribute to ventricular AP duration alterations, especially in the presence of adrenergic activation.

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Building 3-D Models of the Full-Length IKs Cnannel using Computational Techniques

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Background: KCNQ1 (Q1, pore-forming) and KCNE1 (E1, regulatory) associate to form the IKs channel, a critical determinant of QT interval. None of Q1 homology models or Q1/E1 docking models includes the cytoplasmic domain (CD), despite its critical roles in IKs assembly/trafficking/modulation. One major obstacle is the very long (~320 aa) and dynamic Q1 carboxyl terminus (CT). However, sub-regions of CT of Q1 and homologous KCNQ4 have been crystallized, and there is rich information on the functional roles of, and relationships among, sub-regions or residues in CD of Q1/E1. These prompt us to build 3-D models of full-length Q1/E1 (i.e. including CD) using a hierarchical approach.

Methods: (1) Use Robetta server to predict structures of amino-terminus (NT, aa 1-140) and CT (aa 354-676) of Q1. (2) Select Robetta models compatible with existing structural data. (3) Remove flexible loop regions, and dock the helical regions of chosen Robetta models to the Q1 transmembrane homology model. This manual docking procedure is guided by data in the literature. (4) The most favored docking-configurations are triplicated to produce the full-length Q1 models. (5) Dock refined E1 NMR structure to the full-length Q1 model, guided by our disulfide-trapping data and information in the literature. (6) After energy-minimization and removing steric clashes, the systems will be subjected to molecular dynamics (MD) simulations. (7) Analyze the MD trajectories to design disulfide-trapping experiments for model validation or rejection.