Washington University in St. Louis

CARDIAC BIOELECTRICITY AND ARRHYTHMIA CENTER (CBAC)

The Cardiac Bioelectricity and Arrhythmia Center (CBAC) Research Retreat

Friday, May 12, 2006
7:30 AM – 5:00 PM
Whitaker Hall, Washington University in St. Louis, Missouri

Poster Presentation Abstracts

(Poster Presentations are scheduled during the lunch break from 12:20 PM – 2:30 PM, and will be located throughout the Atrium and on the second floor)

290 Whitaker Hall
Campus Box 1097
One Brookings Dr.
St. Louis, Missouri 63130-4899

Tel: (314) 935-7887
Fax: (314) 935-8168
http://cbac.wustl.edu

Cardiac Bioelectricity & Arrhythmia Center (CBAC)

Table of Contents

Ionic Mechanisms of Action Potential Restitution and Short-Term Memory in Cardiac Myocytes: A Simulation Study

Keith F. Decker*, Thomas J. Hund[†], and Yoram Rudy*

**Cardiac Bioelectricity and Arrhythmia Center and Department of Biomedical Engineering, Washington University in St. Louis, Missouri*
[†]Department of Pathology, Washington University School of Medicine, St. Louis, Missouri

Many cardiac arrhythmias, such as those observed in the healing period 4-5 days after myocardial infarction, are thought to be reentrant in nature. Individual cells within the reentrant circuit may be subject to sudden changes in stimulus cycle length (CL). Restitution refers to the initial APD (action potential duration) response of a cell to a change in CL, while short term memory describes the evolution of APD from the initial to the steady state response. Restitution and short term memory are thought to be important determinants of the stability of reentrant circuits, and may be altered in pathophysiological states. The ionic mechanisms underlying these processes are not fully understood, however. In the present study, a recently developed model of the canine ventricular epicardial myocyte was used to investigate ionic mechanisms of APD restitution and short term memory. I_{Kr} and I_{Ks} were found to be the major determinants of the kinetics of APD restitution at short diastolic intervals (DI). Recovery from I_{to} inactivation and increased notch depth were found to be responsible for increased APD at longer DI. Short term memory in the model was predominantly due to the slow adjustment of intracellular Na^+ to changes in CL.

This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li for her administration of lab computing resources.

A Model of L-type Ca^{2+} Channel –Ryanodine Receptor Interaction in the Restricted Space in a Cardiac Ventricular Myocyte

Gregory M. Faber*,† and Yoram Rudy*

**Cardiac Bioelectricity and Arrhythmia Center and the Department of Biomedical Engineering, Washington University, St. Louis, Missouri*
†Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio

L-type Ca^{2+} channels play an important role in the function of cardiac myocytes. Ca^{2+} entry via these channels is the primary source of Ca^{2+} signaling the opening of ryanodine receptors (RyR) that release Ca^{2+} from the sarcoplasmic reticulum (SR) and trigger cell contraction. In addition, the L-type Ca^{2+} current ($I_{Ca(L)}$) is an important determinant of action potential (AP) duration and its rate adaptation during pacing. In this study we present a single channel Markov model of the L-type Ca^{2+} channel that operates in the context of a restricted subcellular space. The channel activates and deactivates in response to voltage and exhibits voltage- and Ca^{2+}-dependent inactivation. The L-type channel Markov model was verified against experimental single channel studies and whole cell macroscopic current studies. The L-type channel Markov model was then tested during an AP clamp at two different rates with APs generated by the Luo-Rudy model of the guinea pig ventricular myocyte. With this model, we were able to compute and display the dynamic occupancy in the various channel states of the L-type Ca^{2+} channels during the AP for slow and fast pacing rates.

This research was funded by NIH-NHLBI Merit Award R37-HL 33342 and RO1-HL 49054. We thank Li Li for her administration of lab computing resources.

Calsequestrin Mutation Results in Spontaneous Calcium Release and Delayed Afterdepolarizations in a Model of the Cardiac Ventricular Myocyte

Gregory M. Faber*,† and Yoram Rudy*

**Cardiac Bioelectricity and Arrhythmia Center and the Department of Biomedical Engineering, Washington University, St. Louis, Missouri*
†*Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio*

Patients with a missense mutation of the calsequestrin 2 gene (CSQN2) are at risk for the development of a catecholaminergic polymorphic ventricular tachycardia. This mutation directly results in a decreased ability of CSQN2 to bind free calcium (Ca^{2+}) in the sarcoplasmic reticulum (SR) and indirectly affects ryanodine receptor (RyR) function via intermediate CSQN-RyR linker proteins modifying SR Ca^{2+} release. We developed and validated Markov models of the L-type Ca^{2+} channel and RyR and incorporated them into the LRd model of a ventricular myocyte. Using this newly developed model we investigate the mutation's effect on SR Ca^{2+} storage, intracellular Ca^{2+} and its indirect effect on membrane potential. In our model at a pacing frequency of 0.5 Hz, myocytes with the CSQN2 mutation exhibited $[Ca^{2+}]_i$ transients with an amplitude 20% lower than control myocytes. Following the addition of 1 μM isoproterenol (Iso), spontaneous Ca^{2+} release and subsequent delayed after-deplarizations (DADs) occurred in myocytes with the CSQN2 mutation. If the resting membrane potential is elevated further (increased Iso), the DADs are able to generate their own action potentials, indicating a possible means by which the mutation is able to trigger a lethal arrhythmia.

This research was funded by NIH-NHLBI Merit Award R37-HL 33342 and RO1-HL 49054. We thank Li Li for her administration of lab computing resources.

Postganglionic nerve stimulation induces temporal inhibition of excitability and unidirectional conduction block in the rabbit sinoatrial node

Vadim V. Fedorov, Halina Dobrzynski, William J. Hucker, Igor R. Efimov.

Cardiac Bioelectricity & Arrhythmia Center, and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

OBJECTIVE: Transient increase of vagal tone results in complex inhomogeneous changes of pacemaker excitability in the sinoatrial node (SAN) region which can induced different arrhythmogenic events.

METHOD: We used optical mapping and postganglionic nerve stimulation (PNS) to investigate the mechanisms of vagally-induced changes of activation pattern in the rabbit SAN (n=17).

RESULTS: Normal excitation originated in the center of SAN with a of cycle length (CL) of 405±14 ms, spread anisotropically along the crista terminalis (CT), and failed to conduct toward the septum. PNS (400-800ms) caused a transient CL slowing by 74±7% and the leading pacemaker shifted inferiorly (78%) or superiorly (22%) from the center of the SAN by 2-10mm along the CT. In the intercaval region, between the SAN center and the septal block zone, PNS induced a transient $9\pm1mm^2$ region of hyperpolarization and inexcitability. The first spontaneous or paced excitation following PNS could not enter this region for 500-1500ms. However, in two preparations, post PNS-induced depolarization triggered spontaneous excitation near the block zone - the region which was previously inexcitable. In one preparation, the block zone region was responsible for a stimulation-induced sustained re-entry. Nadolol (2μM) did not prevent PNS-induced hyperpolarization and inexcitability of the pacemaker cells. We identified by immunolabeling the SAN as neurofilament 160 positive, but connexin 43 negative region (n=5) which has a 6 fold higher level of choline acetyltransferase (ChAT) than CT. Immunolabeling revealed that the PNS-induced inexcitable region is located in the area between the SAN center and the block zone, and has a 2-fold higher density of ChAT than CT.

CONCLUSION: We imaged for the first time that the PNS resulted in transient loss of pacemaker cell excitability in the intercaval region between the center of the SAN and the block zone. We propose that this phenomenon represents unidirectional entrance block in the latent pacemaker area, which may play an important role in cholinergic-induced extrasystolic activity and arrhythmogenesis.

Action Potential Prolongation and Ionic Current Remodeling In Transgenic Mice Overexpressing Sarcolemmal K_{ATP} Subunits

Thomas P. Flagg* and Colin G. Nichols*,†

**Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri*
†Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

We recently demonstrated that overexpression of either an ATP-insensitive Kir6.2[ΔN30,K185Q] mutant (*mut*Kir6.2) or SUR1 subunit in the heart under the transcriptional control of the αMHC promoter suppresses the functional expression of sarcolemmal K_{ATP}, that appears to result from unequal subunit expression. In order to restore K_{ATP} density, we generated a number of double transgenic mouse (DTG) lines that overexpress both SUR1 and mutKir6.2 transgenes. No DTG animals expressing both transgenes at a high level were obtained, however, DTG mice that express one transgene at a high level and the other at a lower level display an increased frequency of arrhythmias and die prematurely. To investigate the molecular mechanisms underlying the phenotype, we determined the electrophysiological profile of DTG and wild type animals. When compared to wild type, peak I_{to} current in DTG ventricular myocytes is significantly *reduced* (32.62 ± 11.84 vs. 65.36 ± 11.84 pA/pF at V_m = +40 mV) while peak I_{Ca} is significantly *increased* (-12.40 ± 1.88 vs. -5.38 ± 0.53 pA/pF at V_m = -5 mV). In combination, these changes lead to significant prolongation of the cardiac action potential (47.2 ± 8.4 vs 19.9 ± 2.8 ms APD_{90}). These results further demonstrate the ion channel remodeling that occurs when mutant K_{ATP} subunits are overexpressed in the myocardium. It remains to be determined whether the cellular changes described here are the trigger for or result from the chronic arrhythmias in the DTG animals.

Regulation of the Cell Surface Expression of Kv4 Encoded $I_{to,f}$ Channels in Ventricular Myocytes

Nicholas Foeger*, Haodong Xu*, Bin Ye*, and Jeannne Nerbonne*,†

**Department of Molecular Biology and Pharmacology, Washington University in St. Louis, Missouri*
†Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

Email: Foegern@msnotes.wustl.edu

Voltage-gated K^+ (Kv) channels are the primary determinants of mammalian myocardial action potential repolarization. Regulation of the biophysical properties and the cell surface expression (i.e. density) of Kv channels is essential for the proper functioning of the heart. Although studies in heterologous expression systems suggest that multiple mechanisms (transcriptional, translational, post-translational) can affect Kv channel surface expression, little is known about the physiologic relevance of these control mechanisms. The rapidly activating, rapidly inactivating, i.e. transient, Kv current ($I_{to,f}$) that is responsible for phase 1 repolarization, for example, is differentially expressed in ventricular myocytes. Biochemical studies suggest that $I_{to,f}$ channels are encoded by the co-assembly of Kv channel pore-forming (α) subunits of the *shal* subfamily (Kv4.x) with accessory subunits. Recently, we have shown that $I_{to,f}$ is eliminated in Kv4.2 targeted deletion (Kv4.2-/-) mice. The Kv4.2-/- background, therefore, presents a unique system in which to probe the molecular mechanisms controlling cell surface expression of $I_{to,f}$ channels. To explore directly the role of a putative endoplasmic reticulum (ER) retention motif in the N-terminus of Kv4.2, a mutant Kv4.2 (Kv4.2 AAA), in which the RKR sequence at residues 35-37were replaced by three alanines, was generated. This construct was subcloned into an adenoviral shuttle vector with EGFP and subsequently introduced into Kv4.2-/-myocytes. Voltage-clamp recordings obtained from EGFP positive myocytes revealed that the voltage-gated outward K^+ current densities are significantly higher in Kv4.2-/- cells expressing Kv4.2 AAA compared with cells expressing EGFP alone or EGFP plus wild type Kv4.2. Although the properties of the Kv4.2 AAA currents do not appear to be identical to $I_{to,f}$, these preliminary experiments suggest a role from the RKR motif in regulating Kv4.2 encoded $I_{to,f}$ channels.

Noninvasive Electrocardiographic Imaging (ECGI) : Application of a Higher Order Interpolation Scheme and Preliminary ECGI Data in Patients With Wolff Parkinson White (WPW) syndrome

Subham Ghosh[1,2], Yong Wang[1,2], Pamela Woodard[1,4], Timothy Smith[1,5], Edward Rhee[1,3] and Yoram Rudy[1,2,3,4,5]

[1]Cardiac Bioelectricity and Arrhythmia Center, and [2]Department of Biomedical Engineering, Washington University in St. Louis, Missouri
[3]Department of Pediatrics, [4]Department of Radiology, and [5]Department of Medicine, Washington University School of Medicine, St. Louis, Missouri

Electrocardiographic Imaging (ECGI) is a new cardiac functional imaging modality, noninvasively reconstructing epicardial potentials, electrograms and isochrones (activation sequence) from multi-channel body surface potential recordings with 250 electrodes on the torso. The electrode positions and the epicardial geometry are obtained simultaneously from ECG-gated thoracic computed tomography (CT) scans. The procedure involves solving Laplace's equation in the source-free volume conductor between torso and heart. We discretize torso and epicardial surfaces using the Boundary Element Method (BEM) over triangular surface elements to obtain a relationship $\mathbf{A\Phi_E = \Phi_T}$, where Φ_E and Φ_T denote epicardial and torso potentials respectively. We formulate a quadratic interpolation (QI) scheme for potentials over six-noded triangular surface elements for our ECGI problem. We show using examples of paced beats in human subjects, that the new QI scheme can improve accuracy of localization (≤ 10 mm) of initiation points in both right and left ventricles compared to linear interpolation (≤ 18 mm). We also apply ECGI to patients with Wolff-Parkinson-White (WPW) syndrome, prior to ablation procedure, to identify the areas of ventricular pre-excitation noninvasively. ECGI was also performed on these patients several hours after ablation and then a month later, to determine the changes in activation induced by the ablation procedure and to investigate progression of repolarization patterns ("cardiac memory"). Preliminary WPW ECGI data show an immediate change of activation. However, dispersion of repolarization increases immediately after ablation but decreases a month later, demonstrating cardiac memory associated remodeling processes.

This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li, Lina El-Esber and Leonid Livshitz for their help in preparing electrodes for body surface potential mapping and acquisition of data.

PKCε-deficient mice show altered Cx43 phosphorylation and distribution during ischemia and preconditioning

Thomas J. Hund[1], Kathryn A. Yamada[2,5], Richard B. Schuessler[3,5], Jeffrey E. Saffitz[4]

[1]*Department of Surgery, Washington University School of Medicine, St. Louis, Missouri*
[2]*Department of Cardiology, Washington University School of Medicine, St. Louis, Missouri*
[3]*Department of Surgery, and Cardiothoracic Surgery Research Laboratory, Washington University School of Medicine; Department of Biomedical Engineering, Washington University, St. Louis, Missouri*
[4]*Department of Pathology, Beth Israel Deaconess Medical Center, Harvard University*
[5]*Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri*

Email: hundt@wustl.edu

Background: Previous studies have shown that ischemia causes dephosphorylation and translocation of connexin43 (Cx43) from gap junctions to intracellular sites with a time course that mirrors electrical uncoupling. Ischemic preconditioning (PC) prevents dephosphorylation of Cx43, preserves Cx43 signal in gap junctions and delays uncoupling during ischemia. **Methods:** To test the hypothesis that PKCε, activated by PC, phosphorylates Cx43 and thereby prevents its subsequent internalization during ischemia, we subjected isolated Langendorff-perfused hearts from wildtype (WT) mice and mice with germline knockout of PKCε (PKCε-KO) to 30 min of no-flow ischemia with or without PC. Cx43 phosphorylation state was analyzed by immunoblotting using a polyclonal anti-Cx43 antibody that detects phosphorylated and non-phosphorylated isoforms. Cx43 phosphorylation at the PKC sites ser368 and ser262 was analyzed by immunoblotting using anti-Cx43 antibodies specific for each phosphorylated isoform. Total Cx43 immunoreactive signal in gap junctions was measured by confocal microscopy. PKC isoform activation was determined by immunoblotting of cytosolic and membrane fractions from ventricular lysates using anti-PKCε and anti-PKCδ antibodies. **Results:** Cx43 signal decreased by 64% in non-PC PKCε-KO hearts during ischemia. PC failed to preserve Cx43 signal in PKCε-KO hearts (56% reduction). Robust Cx43 phosphorylation at ser368 was observed following ischemia in both PC and non-PC PKCε-KO hearts. Furthermore, the level of ischemia-induced ser368 phosphorylation was much greater in PKCε-KO than in WT (448% of WT) while ser262 phosphorylation was comparable. In WT hearts, ischemia induced translocation of PKCδ from cytosol to membrane fractions, while limited PKCδ translocation was observed. In PKCε-KO, ischemia induced marked translocation of PKCδ to the membrane. **Conclusions:** These results indicate that PKCε preserves Cx43 signal in gap junctions mediated by PC. However, phosphorylation of Cx43 at ser368 is not sufficient to prevent intracellular translocation of Cx43, and must be catalyzed by at least one other isoform probably PKCδ, which is also activated by ischemia and preconditioning.

Calcium and Action Potential Alternans in Cardiac Cells: Simulation Study of Mechanism and Role of CaMKII.

Leonid Livshitz and Yoram Rudy

Cardiac Bioelectricity and Arrhythmia Center and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) is known to modulate its activity in response to the frequency, amplitude and duration of Ca^{2+} transients (CaT) in cardiac cells. Recent experiments show that dysfunction of the calcium subsystem can underlie action potential (AP) alternans that cause cardiac arrhythmias and sudden death. Based on experimental data alone, it is difficult to define the interactions between AP duration (APD), sarcoplasmic reticulum (SR) calcium loading, L-type calcium current and CaMKII signaling during alternans.

To address these issues, a mathematical model of SR calcium release was developed and incorporated into established ventricular myocyte models of the guinea pig and canine, developed in our laboratory. During fast pacing rate above 3.3 Hz, the guinea pig and canine models produce sustained alternans of both the APD and CaT amplitude. Simulated AP and CaT clamp protocols confirm that oscillation of the calcium cycling subsystem is driving the APD alternans in both the canine and guinea pig models. In addition, we show that clamping the level of Ca^{2+} in SR eliminates both CaT and AP alternans. On the other hand, APD modulates CaMKII activity via a positive feedback mechanism by increasing intracellular Ca^{2+} loading and CaT. An increase of the CaMKII activity shifts the onset of CaT and APD alternans to slower frequencies, whereas decrease of the activity can suppress CaT and APD alternans, thereby exerting an arrhythmogenic effects. Unfortunately, decrease of CaMKII activity converts the normal positive force-frequency dependence to a negative dependence and eliminates frequency dependent acceleration of relaxation (FDAR) which compromises cardiac function. In conclusion, the model simulations show dual effects of the CaMKII regulatory pathway on cardiac cell function. CaMKII underlies the positive force-frequency and FDAR, enhancing cardiac performance. On the other hand, increased CaMKII activity promotes CaT and AP alternans that can provoke deadly cardiac arrhythmias.

This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li for her administration of lab computing resources.

Molecular Mechanisms Underlying Alterations in $I_{to,f}$ in Murine Left Ventricular Hypertrophy

Celine Marionneau*, Sylvain Brunet*, Huilin Li* and Jeanne Nerbonne*,†

**Department of Molecular Biology and Pharmacology, Washington University, St. Louis, Missouri*
†Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

Voltage-gated K^+ (Kv) channel remodeling is a common feature of left ventricular hypertrophy (LVH). Although previous studies have reported alterations in the functional expression of repolarizing Kv channels, particularly the fast transient outward ($I_{to,f}$) channels, in the hypertrophied myocardium, the molecular mechanisms underlying remodeling are poorly understood. Ongoing experiments are focused on exploring the mechanisms of $I_{to,f}$ channel remodeling in a model of left ventricular hypertrophy (LVH) induced by transverse aortic constriction (TAC). Prior to physiological and molecular experiments, LVH was assessed by non-invasive echocardiography, and subsequently confirmed by LV mass determinations. Voltage-clamp recordings revealed a significant increase in mean ± S.E.M. whole-cell membrane capacitance (C_m) in LV myocytes from TAC, as compared with sham-operated, animals. Mean ± S.E.M. peak outward Kv current densities, as well as the densities of $I_{to,f}$, are reduced significantly in LV cells from TAC animals. Further experiments revealed marked regional differences in the response to pressure overload. Mean ± S.E.M. C_m, for example, is greater in TAC LV cells from the endocardium than the epicardium. Mean ± S.E.M. $I_{to,f}$ densities, however, are reduced significantly only in epicardium LV cells from TAC animals. These observations reveal that the cellular hypertrophy and reduction in $I_{to,f}$ density are distinct cellular responses to pressure-overload. In addition, quantitative RT-PCR revealed marked reductions in expression levels of genes encoding $I_{to,f}$ subunits Kv4.2 (-53.3 ± 3.6 %), Kv4.3 (-33.6 ± 4.2 %) and KChIP2 (-52.9 ± 3.9 %) in whole LV of TAC animals. Ongoing experiments are focused on examining regional differences in mRNA and protein expression levels of Kv channel α and accessory (β) subunits as well as of other regulatory proteins that may contribute to the formation of functional $I_{to,f}$ channels. In addition, proteomic capabilities are now being developed in the lab in efforts focused on identifying new binding partners of Kv4.2 α subunits. Together, these investigations will provide fundamentally important new insights into the molecular mechanisms involved in the generation of ventricular $I_{to,f}$ channels and into the mechanisms underlying functional remodeling of these channels in myocardial hypertrophy.

Autonomic Control and Cell to Cell Communication in the Human Atrio-Ventricular Junction

Megan L. McCain and Igor R. Efimov

Cardiac Bioelectricity & Arrhythmia Center, and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

The atrio-ventricular (AV) junction serves as the only electrical connection between the atria and the ventricles, therefore playing a critical role in the conduction of electrical signals from the sino-atrial (SA) node to the ventricles. The AV node is also responsible for slowing the rate of electrical propagation between the atria and ventricles, providing adequate time for the ventricles to fill before contracting. This property of the AV node is precisely regulated by the autonomic nervous system. Because of its unique functions, the AV node is different from the normal myocardium, having profoundly heterogeneous distribution of both innervation and connexins. Connexins are the proteins responsible for cell to cell coupling, and their expression patterns in the AV node are unique and contribute to its distinctive electrical properties. The AV node has been expensively studied in animal models. However, little basic research has been done in the human heart.

Methods and results: We studied the AV junction from a normal human heart. Masson's trichrome histological sections were used to reconstruct the 3D morphology of the AV junction. Immunohistochemistry was used to determine the density of Connexin 40, Connexin 43, myocytes, fibroblasts, nerve fibers, and sympathetic nerve fibers. We found that connexins are distributed in heterogeneous pattern in the AV junction, which probably contributes to the unique electrical properties of the area. The His bundle was found to express both Cx43 and Cx40, while the AV node was found to have a lower expression of both Cx43 and Cx40. It was also found that the expression of nerve fibers in both the His bundle and AV node are significantly increased compared to the atrial and ventricular myocardium, relating to the increased degree of autonomic control in the AV junction. We investigated colocalization of connexins and vimentin and α-actinin, testing the hypothesis which suggests that myocytes could be electrically coupled with fibroblasts. This study found evidence that myocytes may be coupled to fibroblasts in the atrial approaches to the AV junction.

Conclusions: The human AV junction has heterogeneous expression of connexins and markers of autonomic nervous system, which is required for its unique electrophysiological properties.

A Statistical Approach for Ion Channel Conductance

Ali Nekouzadeh and Yoram Rudy

Cardiac Bioelectricity and Arrhythmia Center and Department of Biomedical Engineering, Washington University in Saint Louis, Missouri

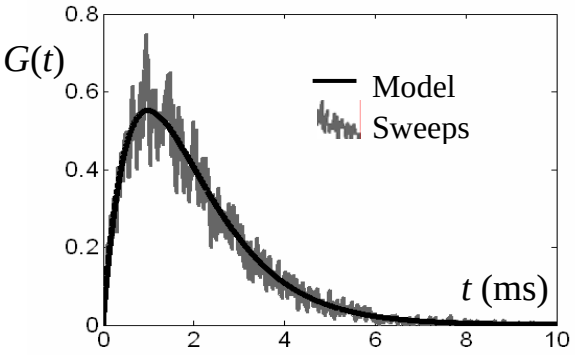
Introduction: Macroscopic Ion channel conductance at the whole-cell level can be derived by summation of the stochastic sweeps of individual channel gating. The focus of this work is to determine how the statistical properties of single channel sweeps govern the shape of the overall cell conductance and which statistical properties of single-channel gating can be derived from the macroscopic conductance.

Method: A simulation program was used to generate one thousand single channel sweeps in response to a step change in membrane voltage. The summation of these sweeps provides the macroscopic conductance at the whole-cell level.

Model: It can be shown that the following Probability Density Functions (PDFs):
M(*t*), PDF of the opening incidents time
D(*t*), PDF of the open-state duration
can predict the ion channel conductance, *G*(*t*) at the whole-cell level.

G(*t*) is related to *M*(*t*) and *D*(*t*) through equation $G(t) = P(t) - M(t) * Q(t)$, where $Q(t) = \int_0^t D(\xi)d\xi$ is the cumulative density function of *t*, $P(t) = \int_0^t M(\xi)d\xi$ is the cumulative density function of *M*(*t*), and * is the symbol of convolution integral.

M(*t*) can be found from *G*(*t*) by assuming exponential distribution with average τ for *D*(*t*),using the equation $M(t) = G'(t) + \frac{G(t)}{\tau}$.



Results: Figure shows the summation of single channel sweeps (grey lines) versus the prediction of the model for *G*(*t*) (black curve).

Acknowledgement: This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li for her administration of lab computing resources.

A Mathematical Model of the Non-failing Human Ventricular Action Potential

Thomas J. O'Hara IV*, László Virág[†], András Varró[†] and Yoram Rudy*

**Cardiac Bioelectricity & Arrhythmia Center (CBAC) and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri*
[†]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary

Different animal models and computer models on different species give quantitatively, and occasionally mechanistically different answers to causal questions related to arrhythmogenesis. Therefore, the utility of an accurate and thoroughly validated human ventricular action potential (AP) model is great. After all, it is the health of humans that medical science aims to improve. A few attempts have been made to mathematically model the human ventricular AP. However, the models fail to accurately reproduce human physiology due to large gaps in the data set upon which they are based. Where human data are missing, the models borrow heavily from animal and heterologous expression system data, rendering them neither human, nor animal, severely undermining their predictive power (human specific or otherwise); the most critical aspect of a human model's research value. Even well composed animal models offer more mechanistic insight than such hybrid creations. Making careful use of all published and newly recorded unpublished experiments in the non-failing human ventricle, we are the first to make use of a complete and fully human specific, non-failing ventricular data set in the construction of a ventricular human AP model. The model offers *de novo* modeling of all major ionic currents including I_{Na}, I_{to1}, $I_{Ca(L)}$, I_{NaCa}, I_{Kr}, I_{Ks} and I_{K1}. Potassium, calcium and sodium handling processes as well as the calcium-calmodulin dependent protein kinase II pathway have been incorporated. All are based exclusively on data from non-failing human ventricular whole-cell patch clamp experiments.

This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li for her administration of lab computing resources.

The I_{Ks} Channel: Structure, Function, and Disease

Hua Pan, **Dick Wu**, and Jianmin Cui

Cardiac Bioelectricity & Arrhythmia Center, and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

Email: hpan@seas.wustl.edu
dw4@cec.wust.edu

The I_{KS} channel, in conjunction with the I_{Kr} channel, repolarizes the myocardium following depolarization by sodium and calcium channels. I_{Ks} is composed of two subunits, KCNQ1 and KCNE1. KCNQ1 alone can form a functional channel. However, the addition of the KCNE1 accessory subunit significantly alters the properties of the channel. Numerous mutations in either subunit can compromise the function of I_{Ks} resulting in long QT syndrome.

We have characterized a mutation of the acidic residue in the second transmembrane helix, E160Q. The mutant KCNQ1 channel exhibits slower activation kinetics when compared to the wild type KCNQ1 channel, resembling that of I_{Ks}. Also, there is no inactivation in the mutant channel, a feature that is prominent in the wild type KCNQ1 channel in the form of a “tail hook” at the beginning of deactivation. The removal of inactivation is a hallmark of the I_{Ks} channel. The coexpression of the mutant channel with KCNE1 does not produce a channel with greater current amplitudes compared to the mutant KCNQ1 channel alone. In contrast, significant current amplification is observed in wild type I_{Ks} currents when compared to KCNQ1 currents.

Our findings suggest that E160 is important to channel function. Research on Shaker and eag channels have identified the S2 and S4 transmembrane segments as components of the voltage sensor that changes conformations to open and close the channel in response to changes in membrane potential. Similar to this mechanism, our results suggest that the glutamic acid in S2 may interact with arginines in S4 in the KCNQ1 channel. By neutralizing the charged amino acid at E160, these interactions are disrupted resulting in a channel that is more difficult to open, hence with slower activation kinetics.

Noninvasive Electrocardiographic Imaging for Cardiac Electrophysiology and Arrhythmia

Charulatha Ramanathan, Raja N Ghanem, Ping Jia, Kyungmoo Ryu, and Yoram Rudy
Nature Medicine 2004; 10: 422 - 428

Over 7 million people worldwide die annually from erratic heart rhythms (cardiac arrhythmias), and many more are disabled. Yet there is no imaging modality to identify patients at risk, provide accurate diagnosis and guide therapy. Standard diagnostic techniques such as the electrocardiogram (ECG) provide only low-resolution projections of cardiac electrical activity on the body surface. Here we demonstrate the successful application in humans of a new imaging modality called electrocardiographic imaging (ECGI), which noninvasively images cardiac electrical activity in the heart. In ECGI, a multielectrode vest records 224 body-surface electrocardiograms; electrical potentials, electrograms and isochrones are then reconstructed on the heart's surface using geometrical information from computed tomography (CT) and a mathematical algorithm. Shown here are examples of ECGI application during focal activation initiated by right or left ventricular pacing and during atrial flutter.

Absence of Mitral Annulus Observations Implies Impaired Diastolic Function

Matt M. Riordan

Department of Biomedical Engineering & Cardiovascular Biophysics Laboratory, Washington University, St. Louis, Missouri

Non-invasive quantitation of left ventricular (LV) diastolic function has traditionally relied on geometric features of the pulsed Doppler echocardiography-determined transmitral flow velocity contour (E-wave). More recently, longitudinal LV function has been assessed via Doppler tissue imaging (DTI) using analogous features of the velocity contour of the mitral annulus. Previously, we proposed and validated damped harmonic oscillation as the paradigm with which to characterize and analyze the motion of the mitral annulus during early diastolic filling (E'-wave). Modeling annular motion as a linear oscillator driven by stored elastic strain energy at the onset of early filling accounts for the reversal in the direction of motion ('ringing' or oscillation) of the annulus after its initial recoil. Moreover, it allows the determination of longitudinal LV stiffness (k'), relaxation (c'), and stored elastic strain (x_o') during early filling. While the reversal of annular motion (toward the apex following its initial atrially-directed motion) has been described qualitatively by others, its importance for longitudinal LV diastolic function characterization has not been fully appreciated. In this study, we examined 37 subjects with and 21 subjects without mitral annulus oscillations via pulsed Doppler echocardiography, DTI, and cardiac catheterization in an effort to characterize these oscillations as a feature of LV diastolic function. Subjects with annular oscillations had better cardiac function based on a variety of global and longitudinal non-invasive and invasively-derived indices. In terms of global indices, subjects with annular oscillations had a higher ejection fraction, shorter isovolumic relaxation time and time constant of isovolumic relaxation, lower relaxation/damping (c), and a lower peak atrioventricular gradient (driving force, kx_o). In terms of longitudinal indices, subjects with annular oscillations had a higher peak E'-wave velocity, diastolic lateral annular excursion, decreased E/E', decreased relaxation/damping (c') and stiffness (k'), and increased stored elastic strain (x_o') and strain energy ($1/2k'x_o'^2$). These results demonstrate that the absence of oscillations of the mitral annulus implies impaired diastolic function. Furthermore, comparison of the magnitudes of various longitudinal and global diastolic function indices in each group suggests that impaired LV longitudinal function is compensated for by other spatial modes in an effort to preserve global LV diastolic function.

Mechanisms of unpinning and termination of ventricular tachycardia

Crystal M. Ripplinger, Valentin I. Krinsky, Vladimir P. Nikolski, Igor R. Efimov

Cardiac Bioelectricity & Arrhythmia Center, and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

High energy defibrillation shock is the only therapy for ventricular tachyarrhythmias. However, due to adverse side effects, lowering defibrillation energy is desirable. We investigated the mechanisms of unpinning, destabilization, and termination of ventricular tachycardia (VT) by low energy shocks in isolated rabbit right ventricular preparations (n = 22). Stable VT was initiated with burst pacing and optically mapped. Monophasic “unpinning” shocks (10 ms) of different strengths were applied at various phases throughout the reentry cycle. In 8 of 22 preparations, antitachycardia pacing (ATP: 8-20 pulses, 50-105% of period, 0.8-10 mA) was also applied. Termination of reentry by ATP was achieved in only 5 out of 8 preparations. Termination by unpinning occurred in all 22 preparations. Rayleigh’s test showed a statistically significant unpinning phase-window, during which reentry could be unpinned and subsequently terminated with E_{80} (magnitude at which 80% of reentries were unpinned) = 1.2 V/cm. All reentries were unpinned with field strengths at or below 2.4 V/cm. Unpinning was achieved by inducing VEP and secondary sources of excitation at the core of reentry. Optical mapping revealed the mechanisms of phase-dependant unpinning of reentry. These results suggest that a 20-fold reduction in energy could be achieved compared to conventional high energy defibrillation and that the unpinning method may be more effective than ATP for terminating stable, pinned reentry in this experimental model.

The GEPD mutation causes allosteric changes in dynamics of the AC region in BK_{Ca} channels

Akansha Saxena*, Gayathri Krishnamoorthy*, Jianmin Cui*,†, and David Sept*

**Department of Biomedical Engineering and the Center for Computational Biology, Washington University, St Louis, Missouri*
†Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

Ion channels are membrane spanning proteins that allow ions to pass in and out of the cell thereby enabling the cell to generate an electric signal. In order to maintain the proper membrane potential, the opening and closing of these channels must be tightly controlled through a process called gating. BK_{Ca} channels are large conductance K^+ channels that are widely expressed in smooth and skeletal muscle, glandular and neuronal tissues. The gating of these channels is controlled by voltage, intracellular Ca^{+2} and Mg^{+2}. The Ca^{+2} dependent gating transition of these channels may involve a conformational change in the gating ring, a cytosolic domain with a similar structure as found in the archeon Ca^{+2} activated K^+ channel, MthK. Studies have revealed that a small area called AC region, the N-terminus of the gating ring, is important in coupling Ca^{+2} binding to channel opening, and the conserved DRDD motif in the AC region is thought to contain the putative Ca^{+2} binding site. Most notably, recent work has shown that a single point mutation changing DRDD to DRGD acts as the gain of function mutation with respect to Ca^{+2} sensitivity and was determined to be directly linked with Epilepsy and Paroxysmal Dyskinesia (GEPD mutation). In order to determine the molecular level mechanism of gating and the effect of point mutations at this position, we have built homology models of both wildtype and mutant AC regions and performed long molecular dynamics simulations on them. Analysis of our trajectories indicates that allosteric effects act through the AC region, coupling changes in the DRDD loop with the movement of two alpha helices directly adjacent to the pore region. Our results and predictions match very well with parallel experimental studies which indicate that the conformational change of AC region is pivotal for change in Ca^{2+} sensitivity of the channel.

Derivation of a Load Independent Index of Diastolic Function

Leonid Shmuylovich* and Sándor Kovács[†]

**Physics Department and Cardiology Internal Medicine, Washington University in St. Louis, Missouri*
[†]Department of Biomedical Engineering, Department of Physics, and Cardiology Internal Medicine; and the Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

More than 30 years ago Suga and Sagawa used the time varying elastance paradigm to propose and validate maximum elastance (E_{max}) as a load-independent systolic function index. E_{max} successfully decoupled loading from intrinsic contractility of the ventricle. However, even though Doppler-echocardiography is the preferred method of non-invasive diastolic function (DF) assessment, the available array of echo-derived indexes are all load-dependent. In fact, despite attempts, no studies have derived and validated an invasive or non-invasive, load independent index of DF (LIIDF). Therefore current clinical assessment of intrinsic DF is confounded by changes in extrinsic load state. We used a quantitative, kinematic model-based approach to predict the existence of, and derive a LIIDF. The (dimensionless) load independent dynamic diastolic efficiency index M is derived and validated as the slope of the peak-driving force ($kx_o \propto$ peak atrio-ventricular gradient) to peak velocity ($cE_{peak} \propto$ peak resistive force) relation. To validate predicted load-independence, load was varied physiologically by tilt-table via head up, supine, and head down positioning in 16 normal, healthy volunteers. For the group, linear regression of kx_o vs. cE_{peak} yielded $kx_o = M(cE_{peak}) + B$ $r^2 = 0.98$; M=1.37, B=4.49. We conclude that the dynamic diastolic efficiency M successfully decouples intrinsic diastolic function from extrinsic preload state.

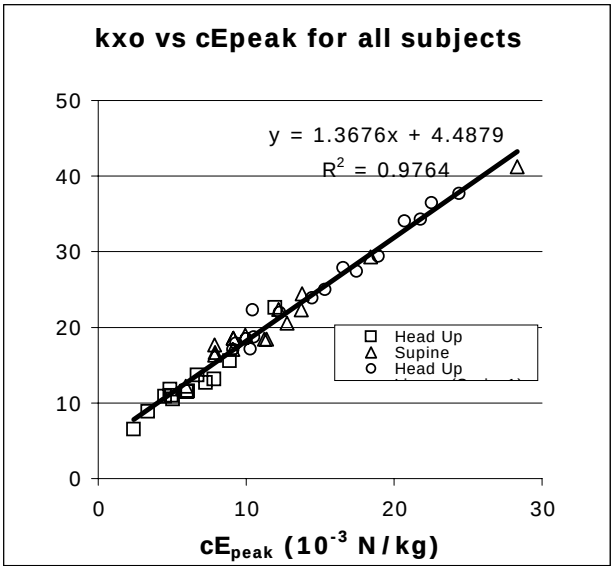


Figure 1: kx_0 vs cE_{peak} plot for all subjects at different preload states. Reported values represent average values of 5 beats defining kx_0 and cE_{peak} for each subject at each preload state.

Subunit Interaction Determines I_{Ks} Participation in Cardiac Repolarization

Jonathan Silva and Yoram Rudy

Cardiac Bioelectricity & Arrhythmia Center, and the Department of Biomedical Engineering, Washington University in St. Louis, Missouri

The role of I_{Ks}, the slow delayed rectifier K^+ current, in cardiac ventricular repolarization has been a subject of debate. We develop a detailed Markov model of I_{Ks} and its α-subunit KCNQ1 and examine their kinetic properties during the cardiac ventricular action potential (AP) at different rates. We observe that interaction between KCNQ1 and KCNE1 (the β-subunit) confers kinetic properties on I_{Ks} that make it suitable for participation in AP repolarization and its adaptation to rate changes, in particular the channel develops an available reserve (AR) of closed states near the open state that can open rapidly on demand. Due to this property, I_{Ks} can function as a repolarization reserve when I_{Kr}, the rapid delayed rectifier, is reduced by disease or drug and prevent excessive AP prolongation and development of arrhythmogenic early afterdepolarizations.

This research was funded by F31-HL68318 (J.S.) and NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (Y.R.). We thank Li Li for help with administration of lab computing resources.

Slow ventricular conduction after ICD shock: Implications of Electroporation

Larisa Tereshchenko[1], Karl Zelik[2], Mitchell Faddis[1,3], Jane Chen[1], Marye Gleva[1], Timothy W. Smith[1,3], Phyllis Stein[1], Peter Domitrovich[1], Bruce Lindsay[1,3], Igor R. Efimov[2,3]

[1]*Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri*
[2]*Department of Biomedical Engineering, Washington University, St. Louis, Missouri*
[3]*Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri*

Previous experimental studies showed that electroporation can selectively affect small trabeculated structures and bundles of the conduction system of the heart (Al-Khadra, 2000). This could result in transient suppression of excitability within the conduction system of the heart and conduction block (Yabe et al. 1990). While high intensity shocks are used routinely to terminate ventricular arrhythmias, clinical data about myocardial tissue responses to large currents and electroporation after ICD shocks are limited. We studied the effect of ICD rescue shock on the width of intracardiac electrogram as a surrogate of ventricular conduction time. Sixty patients (mean age 61.4±13.5 years, 46 men) underwent ICD implantation for primary (75 %) or secondary (25%) prevention of SCD. Ischemic cardiomyopathy was diagnosed in 43 (72%) patients and non-ischemic - in 17 (28%). A transvenous ICD device (Guidant Inc., St. Paul, MN) was implanted in all patients: single-chamber in 42 (70%) and dual-chamber in 14 (23%) patients. Ventricular fibrillation (VF) was induced with a shock-on-T-wave protocol. Ten-second electrograms (EGM) before VF onset, during VF event and after shock were extracted during ICD interrogation; 79 post-shock EGM recordings were considered eligible. EGM analysis was done using customized LabVIEW software. Only sinus ventricular-sensed (VS) beats were included in the analysis. Ten consecutive beats before VF induction were compared with paired consecutive sinus post-shock beats. Initial width of intracardiac EGM (WIE_i) was measured as the time duration from the onset of QRS on far-field EGM to the peak ventricular spike on RV near-field EGM (Figure 1). The first 2 seconds after shock were excluded from analysis to avoid the effect of lead polarization. **Results:** The mean of WIE_i in ventricular sensed (VS) beats during 10 seconds after ICD shock was significantly higher than in 10 control VS beats (34.7±12.9 ms vs 28.1±10.9 ms, $p<0.0001$). Paired T-test also revealed significant differences for all 10 beats. Widening of intracardiac EGM was observed in 69 out of 79 eligible for analysis post-shock EGM recordings (87.3%). In about half of them (30 cases, 43%) ventricular conduction did not restore during 10 seconds after shock. WIE_i was significantly longer if the applied ICD shock was higher than 10 J/m^2 (38.4 ± 13.7 ms vs. 27.1 ± 5.8 ms; $p=0.006$). **Conclusion:** Transient widening of intracardiac ventricular EGM is common after ICD shocks, which is likely a result of shock-induced transient electroporation.

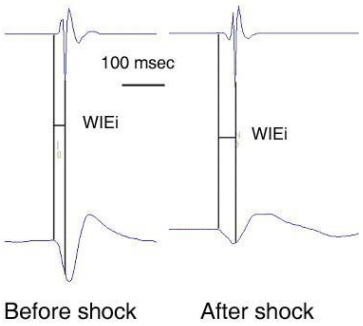


Figure 1*. Near-field (top) and far-field (bottom) EGM recorded during sinus rhythm before and after ICD rescue shock.* WIE_i *before shock 35ms, after shock 45 ms.*

Meshless Method for Noninvasive Electrocardiographic Imaging (ECGI) and Preliminary Application in Human Atrial Fibrillation

Yong Wang[1,2], Subham Ghosh,[1,2] Timothy W. Smith[1,3], Pamela K. Woodard[1,4] and Yoram Rudy[1,2,3,4]

[1]Cardiac Bioelectricity and Arrhythmia Center, and the [2]Department of Biomedical Engineering, Washington University, St. Louis, Missouri
[3]Department of Medicine, and the [4]Department of Radiology, Washington University School of Medicine, St. Louis, Missouri

Electrocardiographic Imaging (ECGI) is a noninvasive imaging modality for cardiac electrophysiology and arrhythmia. ECGI reconstructs epicardial potentials, electrograms and isochrones from body-surface electrocardiograms combined with heart-torso geometry from computed tomography (CT). The method of choice for computing epicardial potentials has been the boundary element method (BEM) which requires meshing the heart and torso surfaces and optimizing the mesh, a very time-consuming operation that requires manual editing. Moreover, it can introduce mesh-related artifacts in the reconstructed epicardial images. Here we introduce the application of a meshless method, the Method of Fundamental Solutions (MFS) to ECGI. This new approach that does not require meshing is evaluated on data from animal experiments and human studies, and compared to BEM. Results demonstrate similar accuracy, with the following advantages: 1. Elimination of meshing and manual mesh optimization processes, thereby enhancing automation and speeding the ECGI procedure. 2. Elimination of mesh-induced artifacts. 3. Elimination of complex singular integrals that must be carefully computed in BEM. 4. Simpler implementation. These properties of MFS enhance the practical application of ECGI as a clinical diagnostic tool. We applied MFS based ECGI to atrial fibrillation patient, and characterized the activation pattern for three consecutive atrial beats. We found that activation starts from the pulmonary veins and inferior/superior vena cava with a different pattern during each beat.

This research was funded by NIH-NHLBI Merit Award R37-HL 33343 and RO1-HL 49054 (to Y.R.). We thank Li Li, Leonid Livshitz and Lina El-Esber for their help in data acquisition.

Scanning the intracellular activation gate of mSlo1 BK_{Ca} Channels

Huanghe Yang*, Lei Hu*, Juqiu Yang*, Akansha Saxena*, Jingyi Shi*,† , and Jianmin Cui*,†

**Cardiac Bioelectricity and Arrhythmia Center, and the Department of Biomedical Engineering, Washington University, St. Louis, Missouri*

†Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri

Ion channels are exquisite molecular machines which control the excitability of cells by regulating ion flux across the cell membrane. The channels are sensitive to the stimulation by cellular signals such as changes in the membrane potential and chemical ligands, and ion flux is eventually switched by the opening and closing transitions (gating) of the channel activation gate. Although several gating mechanisms have been proposed, the molecular nature underlying the gating and the energetic coupling between stimuli and channel gating remains poorly understood. Large conductance Ca^{2+} activated K^{+} channels (BK_{Ca} channels), which are uniquely activated by both membrane voltage and intracellular Ca^{2+}, can be good model systems to explore the molecular motion of the activation gate. In this study, we systematically mutated the residues in the activation gate region of mSlo1 BK channels. We have found that the C-terminus of the inner helices (S6) was important for both voltage and Ca^{2+} activation of mSlo1 channels. The mutations which changed the flexibility the inner helices interrupted the channel gating accordingly. Together with the gating current measurements and the homologue models of BK channels based on the KcsA (closed state) and Kv1.2 (open state) crystal structures, we conclude that the flexibility of the C-terminus of S6 is important for mSlo1 gating and both voltage- and Ca^{2+}-activation pathways finally converged at this region to open the gate.

The Consequence of Time Varying Crossbridge Attachment Rate: Validation in Human, Non-ejecting Left Ventricular Beats

Wei Zhang*, Charles S. Chung*, Sándor J. Kovács*,†

**Cardiovascular Biophysics Laboratory, Cardiovascular Division, Washington University School of Medicine, St. Louis, MO*

†*Cardiac Bioelectricity & Arrhythmia Center (CBAC), Washington University in St. Louis, Missouri*

Contact information:
Wei Zhang
Physics Department, Campus Box 1105
Washington University in St. Louis
One Brookings Drive
St. Louis, MO, 63130
Email: weizhang@hbar.wustl.edu
Phone: 314-454-8337

While the structure of sarcomere is known at the molecular level, the kinematics of crossbridge cycling is not fully understood. The two-state crossbridge framework set up by Huxley is widely accepted; many other models have been developed through the decades. However, no model can explain all the details during a single cardiac cycle under different physiologic states. Experimental results have shown that crossbridge kinetics changes with intracellular and extracellular calcium concentration. It is known that these concentrations change dramatically during the cardiac cycle.

Therefore we postulated that the lumped apparent attachment rate changes respect to time. Organ-level modeling typically assumes that the sarcomere is isometric during isovolumic periods. Thus isovolumic periods can predict the attachment and detachment rates of scarcomeres by assuming binding ratios are equivalent to the tension, and therefore pressure of the organ.

In order to test the hypothesis, we used the model to predict the pressure phase plane (PPP) during non-ejecting left ventricle (LV) premature ventricular contraction (PVC). LV PVC pressure contours from 6 normal healthy patients were analyzed. The differential equation was solved numerically. The time independent attachment and detachment rates can't fit the ratio phase plane (binding ratio R vs. dR/dt) and pressure phase plane (pressure P vs. dP/dt). However, predicted, time-varying binding ratios allowed stronger fit.

We conclude that a time varying attachment rate function provides new insights to crossbridge kinetics and more direct experimental measurements need to be done to fully understand the rate functions.