The Role of Delayed Rectifier Potassium Currents in Human Ventricular Cardiomyocyte Arrhythmogenesis

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Specific Aims

Cardiac arrhythmias are among one of the most widely recognized medical conditions, with the ability to both diminish a patient's quality of life and trigger sudden cardiac arrest, an event that carries approximately a 70% mortality rate (Rakić et al., 2005). Despite its prevalence, little is understood about arrhythmogenic behavior. Cellular irregularities such as early afterdepolarizations have been identified but the mechanisms causing these irregularities are difficult to study *in vitro* and *in vivo* due to the complex nature of the ion balances in cardiomyocytes. *In silico* models have recently assisted in closing the information gap by allowing the ability to generate and test models with multiple and specific parameters. This study will attempt to determine the effect of two ionic currents, the rapid and slow delayed rectifier potassium currents I_{Kr} and I_{Ks}, in generating arrhythmogenic behavior in the O'Hara-Rudy (ORd) model of a human ventricular cardiomyocyte. The identification of early afterdepolarizations at various ratios of I_{Ks}/I_{Kr}, adjusted *in silico*, will allow insight into the contributions of I_{Ks} and I_{Kr} in maintaining an electrophysiologically-stable environment. As demonstrated in the Livshitz-Rudy (LivR) guinea pig ventricular cardiomyocyte model, an I_{Ks} feedback loop rendered that a higher concentration of I_{Ks} was increasingly beneficial to cellular stability. However, guinea pigs present a greater expression and dependence on the I_{Ks} channel, implying that while the same feedback loop may hold true in humans, the effects will be less pronounced. This initial study opens the possibility of either discovering new targets for antiarrhythmic drugs or creating a cell with ideal ratios of each current to discourage cellular arrhythmogenesis in extreme environments. Both offer exciting promises to the future of treating cardiac arrhythmias.

Background and Rationale

Cardiac electrophysiology has presented itself as the most basic means to understanding the synchronized muscle contractions that allow the heart to become an efficient blood delivery system. Action potential (AP) propagation, a wave of electrochemical excitation, creates a depolarized environment for which each individual cellular contraction will contribute to the movement of the entire organ. On a cellular level, the generation, maintenance, and termination of an AP is governed by fluctuations of charge on either side of the cell membrane through ion channels, pumps, and exchangers.

These complex proteins are both voltage-gated and ion selective, allowing for specific ions to move at specific times to create a stable AP dynamic unique to the heart (Jalife et al., 2009b).

The cardiac ventricular action potential is divided into multiple phases, each with distinct changes in polarity and currents (Jalife et al., 2009a). Phase 4 both ends the previous action potential and starts the next AP at a resting membrane potential of -90mV, where K^+ predominates inside the cell and Na^+ outside. Sodium ions from a neighboring cell leaks into the ventricular cardiomyocyte through gap junctions, crossing a threshold potential. Sodium channels open to allow Na^+ to rush inside the cell, rapidly depolarizing it to a positive potential of approximately +40mV. Sodium channels then close and potassium channels open, creating the outward transient potassium current I_{to}, slightly repolarizing the cell. However, as the membrane potential approaches +5mV, calcium channels open and Ca^{2+} stored outside of the cell moves in. The inward calcium current I_{Ca} and the continuing outward movement of potassium, now the delayed rectifier potassium current I_K, balance each other out and the membrane potential remains unchanged. Note that the movement of Ca^{2+} ions marks the cell's physical contraction. Finally, the calcium channels close, allowing the continuing potassium channels to allow full repolarization back to resting membrane potential. Displaced ions are correctly placed by both the sodium-potassium ATPase (NKA) and sodium-calcium exchanger (NCX), creating the inward rectifier current I_{K1}. The interactions of ions and currents creates an action potential that lasts approximately 300ms, allowing enough time for blood to fill each chamber of the heart before being pumped out.

The cardiac action potential is both complex and remarkably resilient. However, interruptions in the AP and AP propagation can lead to unorganized electrical flow down the heart and open the heart to the possibility of arrhythmogenic behavior. Cardiac arrhythmias typically develop at states of bradycardia and tachycardia, where a safe blood pressure cannot be maintained and heart cells fail receive oxygen (Keating & Sanguinetti, 2001). Arrhythmias include but are not limited to atrial fibrillation, pulseless electrical activity, torsades de pointes, ventricular tachycardia, and ventricular fibrillation, the last three being the most dangerous and predisposing to sudden cardiac arrest (SCA). SCA contributes to more than 325,000 adult deaths every year; it is the leading cause of natural death and responsible for half of all heart disease deaths (Cleveland Clinic, 2003). Even within a highly equipped coronary care unit, only 29.2% of patients with witnessed SCA survived to discharge; less than 4% survive an unwitnessed SCA (Rakić et al., 2005). Despite its high mortality rate and increasing prevalence, the underlying mechanisms of arrhythmias are still relatively unknown. AP irregularities such as early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) that make cells contract out-of-beat to trigger arrhythmias have been identified, but even elucidating a complete understanding of these irregularities is difficult given the complexity of the ion interactions within the cell (Weiss et al., 2010). It is generally understood that

changes in the cardiomyocyte that increase the action potential duration (APD) are more likely to incite EADs. These are represented in an ECG as an increase of the QT interval, increasing risk for arrhythmogenesis (Krogh-Madsen et al., 2017).

Studies aimed at understanding the cellular AP irregularities often isolate singular heart cells, cardiomyocytes, deliver electrical impulses to stimulate the cell, and observe the action potential. This method does not provide a reliable means to understand the function of specific ion channels, however, due to the inability to distinguish each channel's contribution to the action potential. *In silico* models, then, have been widely commended as the means to closing this loop of information. These computer models allow researchers to study the heart by controlling parameters and recording results more precisely, in addition to being able to simulate the native environment of the cell for more holistic results (Colquitt et al., 2011).

Traditionally, *in silico* AP models are created from mathematical equations derived from real cell behavior and experimental data; each equation individually describes the activity of a significant contributor before being integrated as one and optimized to fit a target, such as AP form. Hodgkin and Huxley (1952) first mathematically described the initiation and propagation of a neuronal AP in their Nobel Prize winning work. Noble (1962) then modified these equations to model the activity of cardiomyocytes by including I_{K1} and properly delaying I_K kinetics. Later discoveries identified the presence of more ion channels and increased the complexity of previous models. Newer models are suggested to be optimized with genetic algorithms (GA), allowing for more precise manipulation (Groenendaal et al., 2015). Thus, *in silico* models that accurately reflect *in vitro* and *in vivo* results lie as the best means to both understanding arrhythmias to eventually minimize the risk of sudden cardiac arrest.

Cardiac AP models remain one of the most detailed models in systems biology, with models describing various regions of the heart in rabbits, canines, mice, and more recently, humans (Noble et al., 2012). While animal studies are highly beneficial to understanding AP propagation, cardiomyocytes vary between different species and even between different regions of the heart. Many humans models are also based on induced-pluripotent stem cell cardiomyocytes (IPSC-CMs), which have their own limitations in cell maturity and gene expression. Difficulty has arisen in deducing whether the same mechanisms found in these models hold true for mature human cardiomyocytes *in vivo*. Due to these factors, human ventricular cardiomyocyte models such as the O'Hara-Rudy (ORd) model may not reflect the true behavior of cardiomyocytes.

The Livshitz-Rudy (LivR) model was originally used to investigate the effects of I_{Kr} and I_{Ks}, the rapid and slow components of the delayed rectifier potassium current I_K, in a guinea pig left ventricular

cardiomyocyte (Devenyi et al., 2016). The study found that a larger concentration of I_{Ks} helped stabilize guinea pig ventricular AP due a feedback mechanism based on its slow activation kinetics, making it more resistant to EADs even in the face of an increasing action potential duration. However, due to the nature of guinea pig cardiomyocytes and the aforementioned cellular discrepancies, this study will attempt to understand the relationship between

1. Human ventricular cardiomyocytes and guinea pig ventricular cardiomyocytes in their application of results, and
2. I_{Ks} and I_{Kr} in increasing cellular resistance to arrhythmogenic irregularities

Research Questions and Hypotheses

The goal of this study is to explore the effect of two delayed rectifier potassium channels on maintaining human ventricular cardiomyocyte stability. Previous research indicates a regulatory feedback mechanism exists within one of the aforementioned potassium channels, I_{Ks}, in guinea pig ventricular cardiomyocytes. However, interspecies variation of ventricular cardiomyocytes includes fundamental electrophysiological differences in ion channels and dominating currents. Guinea pig hearts are notorious for an upregulation of I_{Ks}. Models, subsequently, show I_{Ks} as the major rate dependent current and drastic changes in APD with I_{Ks} block compared to a human model (O'Hara & Rudy, 2012). To study both interspecies differences and the subsequent effect of I_{Ks} in humans, the O'Hara-Rudy (ORd) human ventricular cardiomyocyte model will be obtained from open-source databases and modified to display both APD90 and detect cellular irregularities. I_{Ks} and I_{Kr} will be placed into varying ratios at which will be tested against a calcium current, I_{CaL}.

Upon investigation, the ORd model does display significantly fewer I_{Ks} channels than the guinea pig model. This implication creates the following hypothesis: the stabilizing effect produced from I_{Ks} regulatory feedback in the guinea pig ventricular cardiomyocyte will hold true for humans but to a much smaller extent. Despite this, determining the existence of this effect will undoubtedly prove useful for future steps in human arrhythmia research; APD dependence on I_{Ks} can identify a new target for antiarrhythmic drugs and APD independence of I_{Ks} will make the creation of an electrophysiologically-stable cell more flexible and achievable.

Research Design and Methods

1. Obtain model of human ventricular cardiomyocyte

Various models have been used to describe human ventricular cardiomyocytes. However, many models lack validation of their behavior due to lack of data. The O'Hara-Rudy (ORd) model, developed

from both previous work and new undiseased human ventricular data, is currently one of the most relevant models. Due to this experimental validation, the ORd model will be used to accurately determine the effect of I_{Ks} and I_{Kr} on arrhythmogenic behavior. The model is an open source tool and will be available from the Rudy Lab code repository.

2. Creation of EAD detection system

The ORd model dictates the prediction of 1000 APs but only displays the last two. Due to this, running the model and observing the presence of an EAD for each display is extremely tedious. In a normal AP, voltage will increase very quickly with depolarization and then only decrease as the cell repolarizes. Therefore after the AP reaches its maximum amplitude, there will always be a negative change in voltage ($-\Delta V$). However, if an EAD occurs, there will be a second depolarization wave as the cell is repolarizing— a sudden positive ΔV. The model will then be modified to identify instances of multiple, sequential $+\Delta V$.

3. Studying IKr/IKs balance

I_{Ks} and I_{Kr} are two components of the same channel, currently only differentiated by speed. Due to this, the channels cannot be studied independently. Instead, they will be measured as a ratio of I_{Ks} to I_{Kr}. These ratios will be carefully determined to maintain a baseline APD. Because models are based off of real behavior, it is to be noted that the prevalence of a current is a result of its environment and cannot be directly changed. In order to reflect a change in both I_{Ks} and I_{Kr}, conductance (G), which governs the amount of ions able to pass through the channel, will be multiplied to get the desired ratios.

4. Testing arrhythmogenic resistance

Longer APDs make the cell more susceptible to EADs. Therefore, EAD resistance will be tested by situating ratios of I_{Ks}/I_{Kr} in environments aiming to increase APD. The duration of the inward L-type calcium current I_{CaL}, a component of I_{Ca}, is proportional to the duration of the overall action potential. Thus, I_{CaL} will be systematically increased at each ratio.

Risk Assessment

No potentially hazardous biological agents or hazardous chemicals, activities, and devices will be used during experimentation. Simulations and data analysis will be conducted on MATLAB & Simulink.

Data Analysis

Resulting simulations from the ORd model will be observed with the addition of a detection method based on ΔV to verify the presence of an EAD. In all instances where an EAD does not occur, APD_{90}, action potential duration at 90% of its full repolarization, will be calculated using MATLAB & Simulink to provide more specific evidence of the behavior of I_{Ks} and I_{Kr}. APD_{90} is a favored parameter due to the subjectivity of the beginning and end of a full AP.

The largest I_{CaL} perturbation a cell can withstand will indicate its resistance to EADs. Higher resistance at a larger I_{Ks}/I_{Kr} ratio will indicate if the cell behavior is due to a greater presence of I_{Ks}. Alternatively, higher resistance at a smaller ratio will indicate a greater presence of I_{Kr}. Statistical significance will be established using a two-way *t* test with $a < 0.005$.

Bibliography

Cleveland Clinic. (2003). Sudden Cardiac Death (Sudden Cardiac Arrest). Retrieved from https://my.clevelandclinic.org/health/diseases/17522-sudden-cardiac-death-sudden-cardiac-arrest

Colquitt, R. B., Colquhoun, D. A., & Thiele, R. H. (2011). In silico modelling of physiologic systems. *Best Practice & Research Clinical Anaesthesiology,25*(4), 499-510. doi:10.1016/j.bpa.2011.08.006

Devenyi, R. A., Ortega, F. A., Groenendaal, W., Krogh-Madsen, T., Christini, D., & Sobie, E. A. (2016). Differential roles of two delayed rectifier potassium currents in the regulation of ventricular action potential duration and arrhythmia susceptibility. *The Journal of physiology, 595*(7), 2301–2317. doi:https://doi.org/10.1113/JP273665

Groenendaal, W., Ortega, F. A., Kherlopian, A. R., Zygmunt, A. C., Krogh-Madsen, T., & Christini, D. J. (2015). Cell-specific cardiac electrophysiology models. *PLoS computational biology, 11*(4), e1004242. doi:10.1371/journal.pcbi.1004242

Hodgkin, A. L., & Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of physiology, 117*(4), 500–544. doi:10.1113/jphysiol.1952.sp004764

Jalife, J., Delmar, M., Anumonwo, J., Berenfeld, O., & Khalifa, J. (2009a). Bioelectricity. In *Basic Cardiac Electrophysiology for the Clinician*(2nd ed., pp. 43-71). Wiley-Blackwell.

Jalife, J., Delmar, M., Anumonwo, J., Berenfeld, O., & Khalifa, J. (2009b). Ion Channels. In *Basic Cardiac Electrophysiology for the Clinician*(2nd ed., pp. 43-71). Wiley-Blackwell.

Keating, M. T., & Sanguinetti, M. C. (2001). Molecular and Cellular Mechanisms of Cardiac Arrhythmias. *Cell*. doi:https://doi.org/10.1016/S0092-8674(01)00243-4

Krogh-Madsen, T., Jacobson, A. F., Ortega, F. A., & Christini, D. J. (2017). Global Optimization of Ventricular Myocyte Model to Multi-Variable Objective Improves Predictions of Drug-Induced Torsades de Pointes. *Frontiers in physiology*. https://doi.org/10.3389/fphys.2017.01059

Noble, D. (1962). A modification of the Hodgkin--Huxley equations applicable to Purkinje fibre action and pace-maker potentials. *The Journal of physiology, 160*(2), 317–352. doi:10.1113/jphysiol.1962.sp006849

Noble, D., Garny, A., & Noble, P. J. (2012). How the Hodgkin-Huxley equations inspired the Cardiac Physiome Project. *The Journal of physiology, 590*(11), 2613–2628. doi:10.1113/jphysiol.2011.224238

O'Hara, T., & Rudy, Y. (2012). Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. American journal of physiology. Heart and circulatory physiology, 302(5), H1023–H1030. doi:10.1152/ajpheart.00785.2011

Rakić, D., Rumboldt, Z., Carević, V., Bagatin, J., Polić, S., Pivac, N., & Avelini-Perković, R. (2005). In-hospital Cardiac Arrest and Resuscitation Outcomes: Rationale for Sudden Cardiac Death Approach. *Croatian Medical Journal,46*(6). Retrieved from http://www.cmj.hr/2005/46/6/16342343.pdf

Weiss, J., Garfinkel, A., Karagueuzian, H., Chen, P., & Qu, Z. (2010). Early Afterdepolarizations and Cardiac Arrhythmias. *Heart Rhythm*. doi:10.1016/j.hrthm.2010.09.017

Dutta, S., Chang, K. C., Beattie, K. A., Sheng, J., Tran, P. N., Wu, W. W., … Li, Z. (2017). Optimization of an In silico Cardiac Cell Model for Proarrhythmia Risk Assessment. *Frontiers in Physiology*, *8*. doi: 10.3389/fphys.2017.00616

O' Hara, T., Virág, L., Varró, A., & Rudy, Y. (2011). Simulation of the Undiseased Human Cardiac Ventricular Action Potential: Model Formulation and Experimental Validation. *PLoS Computational Biology*, *7*(5). doi: 10.1371/journal.pcbi.1002061

No changes were made over the course of this investigation.