Modeling the Impacts of Reduced Sialylation on \( K_V \) Gating in Mouse Ventricular Myocytes

Dongping Du, Hui Yang, Andrew R. Ednie and Eric S. Bennett

Abstract—Action potentials (AP), the net change of transmembrane potentials of cardiac myocytes, are produced by the orchestrated functions of ion channels. The voltage gated K+ channels (\( K_V \)) play a significant role in determining the repolarization of AP. Slight change in \( K_V \) functions can affect the AP duration, and lead to abnormal cardiac electrical activity resulting in life-threatening cardiac events. Cardiac disease has been increasingly involved among patients with congenital disorders of glycosylation, which promotes investigations on how aberrant glycosylation impact cardiac functions. We recently reported that reduced sialylation initiated by the deletion of the sialytransferase ST3Gal4 leads to a reduction in sialylation of ventricular \( K_V \) 4.2 and \( K_V \) 1.5 that contribute to \( I_{to} \) and \( I_{Klow} \), thereby limiting \( K_V \) activity during the AP. However, linking the impact of reduced sialylation on each \( K_V \) to the altered cardiac functions at the cellular level is challenging. In addition, studying the gating mechanisms of each individual \( K_V \) in reduced sialylation is difficult due to the challenges of experimental decomposition of K+ currents, since K+ channels activate at similar range of voltage, and share overlapping kinetics. This present investigation models the K+ currents, i.e., \( I_{to} \), \( I_{Klow} \) and \( I_{ss} \) under the ST3Gal4 knockout and controls, respectively, and predicts the impact of each individual K+ current on repolarizations and APs. The study describes the fine-grained details of specific K+ currents. As such, it enables the interpretation of reduced sialylation modulation dynamics in the gating of each individual K+ channel. This work potentially suggests new pharmaceutical targets for the glycan-altered cardiac diseases and potentially other cardiac disorders.

I. INTRODUCTION

Action potential (AP), the net change of transmembrane potential in a cardiac myocyte during a contraction cycle, is produced by the orchestrated function of ion channels. A slight change in ion channel activity may influence the AP waveform and electrical conduction, thereby potentially lead to severe cardiac disorders. Potassium (K+) currents carrying outward currents in the physiological range of potential are involved in nearly all phase of the AP. K+ currents play essential roles in either holding the resting potential near the K+ equilibrium potential or repolarizing the AP [1]. Therefore, the shape and the duration of the AP are determined by the gating of each K+ channel, and the diverse distribution of K+ channels has important physiological functions [1]. In other word, aberrant K+ channel activities can alter APs, hence, affect the electrical conduction, leading to life-threatening cardiac arrhythmias [2-4].

Voltage-gated K+ (\( K_V \)) channels are one major class of K+ channels, which controls the repolarization and duration of APs. \( K_V \) channels are closed at the resting potential, but activate at different membrane potentials upon repolarization to produce sustained or transient outward currents [1]. These currents are carried by different \( K_V \) isoforms that display distinct physiological and pharmacological properties. For example, the transient outward K+ current (\( I_{to} \)) is produced by the \( K_V \) 4.2/4.3, and is responsible for the early repolarization of the cardiac AP; Studies have found significant reduction of \( I_{to} \) in myocytes from patients with severe heart failure [5, 6].

The reduction in repolarizing outward K+ currents are likely to cause delayed repolarization in cardiac APs, hence lead to prolonged AP durations that are common electrophysiological abnormalities in cardiac myocytes from the failing heart. It has been established that diminish of outward K+ currents presents in human atrial and ventricular myocytes in variety of heart conditions such as heart failure and cardiac arrhythmias [7]. Therefore, investigating the molecular basis of the electrical remodeling of K+ channels has important practical implications for pathological analysis and pharmaceutical designs.

Cardiac ion channels are heavily glycosylated, with up to 30% of a mature protein’s mass comprised of glycan structures. Glycosylation is a ubiquitous cellular process that involves the coordinated activities of hundreds of thousands of unique glycan structures [8]. The dynamic process of glycosylation produces various collections of the glycan structures, which leads to distinctive structures and mechanisms in cells [8-15]. It has been shown that glycan has significant effects on cardiac electrical activities [16]. For example, the carbohydrate residue sialic acid has unique impact on cardiac electrical signaling in that it bears a negative charge at physiologic pH originating from a carboxylic acid at it C1 carbon [17].

Aberrant sialylation could affect the surface potential of cardiac myocytes, which possibly remodels the K+ currents produced by the voltage-gated K+ channels, hence alters the repolarization and causes cardiac abnormalities. Cardiac disease has been increasingly involved among the Congenital Disorder of Glycosylation (CDG) patients, which promotes studies of how reduced glycosylation affects the cardiac electrical signaling across different physical scales such as channels and cells.

This paper integrates in-vitro experiments with the in-silico study to model and predict how reduced sialylation influences
the K+ channel gating as well as the AP waveform in ventricular myocytes. We derived the analytical formulations of the models of voltage-gated K+ channels, and optimally calibrated the computer models to match in-vitro data. Further, we integrated the channel model with the cellular model to predict the impact of reduced sialylation on the AP of ventricular cells. The computer experiments associate the changes at the channel level with the changes at the cellular level, which gives detailed explanations on how reduced sialylation influence cardiac electrical signaling.

The rest of the paper is organized as follows: Section II presents the background knowledge of Reduced Glycosylation and modeling of K+ channel gating. Section III introduces the research methodology. Section IV presents the Materials and Experimental Design. Section V presents experimental results, which is followed by Section VI Conclusions.

II. RESEARCH BACKGROUND

It has been shown that reduced sialylation can impact the ventricular repolarization by modulating the isoforms of cardiac ion channels. The glycans of proteins’ mass in cardiac ion channels are typically terminated by negatively charged sialic acid residues that modulate voltage-dependent channel gating directly. Studies indicate that sialic acids can alter the voltage-gated channel activities in the manners of varying the number/location of sialic acids attached to the subunits of channels [16]. β-Galactosidase α-2, 3-sialytransferase 4 (ST3Gal4) is a type of sialytransferase that is responsible for attaching sialic acids to lipids and/or glycoproteins. We have reported previously that deletion of ST3Gal4 (ST3Gal4−/−) increases the susceptibility to ventricular arrhythmias [18]. The study demonstrated that ST3Gal4−/− contributes to a depolarizing shift in the ventricular voltage-gated Na+ activities and increases the rate of recovery from fast inactivation. We also predicted the impact of ST3Gal4−/− in ventricular cells, i.e., the ST3Gal4−/− likely shortens refractory periods of ventricular cells. As a continued work of the previous investigation, this study aims to test the contributions of ST3Gal4−/− on the K+ activity and the repolarization of ventricles.

In order to answer whether and how reduced sialylation impacts K+ activity and cardiac electrical signaling, in-vitro experiments were conducted to measure and characterize K+ currents. Although we observed changes in ST3Gal4−/− ventricular myocytes and across the ST3Gal4−/− ventricle, understanding mechanistic details of pathological variations in K+ currents and APs is challenging without performing in-silico studies. For example, it is difficult to associate the changes of individual K+ currents at the channel level to the variations at the cellular level. Furthermore, identifying the systematic contribution of each K+ current to the delayed/shortened repolarization in cardiac myocytes is extremely difficult without performing computer experiments. Therefore, there is an urgent need to integrate computer models with experimental data to model the variations of ion channels in reduced sialylation and the resulting changes in ionic currents and APs.

The K+ channels generally produce six different type of K+ currents, i.e., a transient outward current (Ito), a ultra-rapidly delayed rectifier K+ current (IKur), a sustained non-inactivating current (Iks), a rapid delayed rectifier K+ current (IKr), a slow delayed rectifier current (IKs), and a time-independent K+ current (IK1) [11, 19]. The Ito and IKslow are thought to be conducted through the 4 isoforms: Kα4.2/Kβ4.3, Kα1.5 and Kβ2.1, and contribute to a rapid repolarization in mouse ventricles [20-23]. Iks is likely conducted through the non-voltage-dependent channels K2P, which is a “two-pore” type channel [24]. It may be noted that the IKr, IK1 and IKs show very small magnitudes comparing to Ito, IKslow and Iks. Therefore, many in-vitro studies focus on the three major currents while investigating the pathological changes among K+ channels [25]. Although eliminating smaller K+ currents makes the in-vitro study easier, to measure the K+ currents formed by different type of channels remains challenging. The Kα channels activate at similar range of voltage, and share overlapping kinetics, so it is difficult to separate K+ currents experimentally through patch clamp protocols [19, 26]. This present study use mathematical models to simulate the effects of reduced sialylation on each individual currents of Ito, IKslow and Iks, and further predict the corresponding impact on the repolarization in ventricular cells.

The first model of K+ channel in cardiac myocytes was developed by Beeler and Reuter in 1977, which describes the ion current mathematically in terms of Hodgkin-Huxley type equations [29]. Furthermore, Shibasaki proposed a formulation that models the delayed outward rectifier K+ current Ik with a time-dependent activation gate and a time-independent inactivation gate capturing the inward rectification properties of the delayed rectifier current, meanwhile, the conductance was modulated by extracellular K+ concentration [30]. In addition, the 1991 Luo-Rudy Phase I model described three K+ currents, i.e., a time-dependent delayed rectifier K+ current (Ik), a plateau K+ current (Ikp) and a time-independent K+ current (Ik1), based on the data at the time from voltage clamp studies [31]. The phase I model was further improved by reformulating Ica based on the recent experimental data, whereas, the number of K+ currents remained the same except a Na+/K+ pump current was included [32]. With more experimental data available, Bondarenko and Rasmussen derived a model of mouse ventricular myocytes with six K+ currents included [33]. This model detailed the delayed rectifier K+ currents with three individual currents, i.e., a rapid delayed rectifier K+ current (IKr), an ultra-rapidly activating delayed rectifier K+ current (IKur), and a slow delayed rectifier K+ current (IKs), based on the previous efforts [34-39]. In addition, the transient outward K+ current was separated into two currents of rapidly inactivating transient outward K+ current (Itof) and slow-inactivating transient outward K+ current (Itos), where Itof mainly presents in the septum region of ventricles.

In this study, we couple in-silico studies with the wealth of
data from our electrophysiological experiments to model, mechanistically, how reduced sialylation that occurs in the ST3Gal4−/− heart affects $K_v$ channel activity and electrical signaling in the adult mouse ventricle. Indeed, computer models not only overcome practical and ethical limitations in physical experiments but also provide predictive insights into the underlying mechanisms.

III. RESEARCH METHODOLOGY

We previously modeled the transition of molecular states in ST3Gal4−/− Na_v channels and myocytes, showing that glyco-
gene defect impacts Na_v channel activity and leads to shortened refractory periods. In this study, electrophysiological experiments have been conducted to measure how ST3Gal4−/− affects the $K_v$ channel activities and APs of mouse ventricular myocytes. The in-vitro experiments produced two sets of data: one under “control” (physiological, wild-type - WT) conditions, and the other under conditions of reduced sialylation (pathology, ST3Gal4−/−). In this section, we will detail computer models that are used to describe mechanistic details of pathological variations in $K_v$ channels and the AP of ventricular myocyte. With the computer model, changes in ion channels are modeled, and effect on cardiac function at cellular level is predicted.

3.1 Computer Model of Ventricular Myocyte

As aforementioned, the magnitudes of K+ currents are distinctive. To further quantify the contribution of each K+ current to the joint K+ currents, we tested the current density of K+ currents at different clamp voltage (-50mV–50mV) with Bondarenko et al model following the Steady State Activation protocol (Protocols are given in Section IV). The peaks of K+ currents at each clamp voltage are recorded, and plotted in Figure 1. The experiment indicates that $I_{K1}$, $I_{Kr}$ and $I_K$ present very small magnitudes at all clamp voltages of -50mV–50mV. However, $I_{to}$, $I_{Kur}$ and $I_{Kss}$ shows significantly higher magnitudes, especially at the clamp voltage of -20mV–50mV. Therefore, in our simulation we propose to decompose the joint K+ currents measured from in-vitro experiments into the three major currents, i.e., $I_{to}$, $I_{Kur}$ and $I_{Kss}$.

![Figure 1. Current voltage relationship in Bondarenko et al 2004 model.](image)

3.2 Computer Model of $K_v$

Transient outward K+ current ($I_{to}$) contributes to the depolarization of APs. It characterizes the isoforms of the $K_v$ 4.2/3.4.2. Typically, there are two type of transient outward K+ currents, i.e., rapidly inactivating transient outward K+ current ($I_{to,f}$) and slow-inactivating transient outward K+ current ($I_{to,s}$). In this present study, we are investigating the effects of reduced glycosylation on the $K_v$ in apical ventricular cells, where little amount of $I_{Kto,s}$ is presented, so we only modeled a single transient outward current, i.e., $I_{to}$. Bondarenko et al model describes the gating of $I_{to}$ channels with an activation and an inactivation; the gating variables are calculated with ordinary differential equations [26]. In our experiments, to reduce the computational complexity and to improve the efficiency of the model calibration, we describe the K+ currents analytically as follows.

$$I_{to} = G_{Kto}a_{to}^3i_{to}(V - E_K)$$

$$a_{to}(t) = (a_{to}(0) - a_{ss, to})e^{-\frac{t}{\tau_{ato}}} + a_{ss, to}$$

$$i_{to}(t) = (i_{to}(0) - i_{ss, to})e^{-\frac{t}{\tau_{ito}}} + i_{ss, to}$$

where $G_{Kto}$ is the maximum whole cell conductance (mS/uF), $a_{to}(0)$ and $i_{to}(0)$ are the initial values, $a_{ss, to}$ and $i_{ss, to}$ are the steady state activation and steady state inactivation, $\tau_{ato}$ and $\tau_{ito}$ are activation and inactivation time constants. The formulations of steady states activation, steady state inactivation, time constants are listed in Table I.

In this study, we separate the ultra-rapidly delayed rectifier K+ current $I_{Kur}$ (denoted as $I_{Kslow}$ in this paper) into two currents, i.e., $I_{Kslow1}$ and $I_{Kslow2}$. The two currents share the same activation variable because the steady state activations measured from the two channels are identical, however, the inactivation are modeled differently since the in-vitro steady state inactivation shows a bi-Boltzmann shape. The $I_{Kslow1}$, j = 1, 2, is described by the following differential equations:

$$I_{Kslow1,j} = G_{Kslow,1,j}a_{ur}i_{ur,1}(V - E_K), j = 1, 2$$

$$a_{ur}(t) = (a_{ur}(0) - a_{ss, 1})e^{-\frac{t}{\tau_{uar}}} + a_{ur}$$

$$i_{ur,1}(t) = (i_{ur,1}(0) - i_{ss, 1})e^{-\frac{t}{\tau_{ur1}}} + i_{ur}$$

$$i_{ur,2}(t) = (i_{ur,2}(0) - i_{ss, 2})e^{-\frac{t}{\tau_{ur2}}} + i_{ur}$$

where $G_{Kslow,1,j} = 1$ is the maximum whole cell conductance (mS/uF), $a_{ur}$ and $i_{ur, j = 1, 2}$, are activation and inactivation gates. Figure 2 shows simulated steady state activations (SSA), steady state inactivation (SSI), current densities, and time constants of $I_{Kslow1}$ and $I_{Kslow2}$. The SSA and SSI of $I_{Kslow}$ (i.e., the sum of $I_{Kslow1}$ and $I_{Kslow2}$) yields to the optimal fit to our in-vitro data (See Section V). The SSA and SSI of $I_{Kslow2}$ are similar to the experimental results from [42] (Figure 2 a&b). SSA is calculated by depolarizing the cells to -50mV to 50mV with 10 increment for 4.5ms. In SSI experiments, 10s depolarizing pulse from -110mV to 0mV and from -80mV to 0mV were applied to $I_{Kslow1}$ and $I_{Kslow2}$ channels respectively for 10s, which are followed by a 4.5s pulse to 40mV. The conductance of $I_{Kslow1}$ and $I_{Kslow2}$ are chosen to be 0.0276 mS/uF and 0.1036 mS/uF to be close to the experimental observations from [42]. The current-density relationships are shown in Figure 2c. The magnitude of
$I_{Kslo2}$ are close to the observations in [42]. The inactivation time constants were obtained by fitting the decaying portion of current traces to an exponential equation. The time constants of $I_{Kslo1}$ and $I_{Kslo2}$ are also closest to the decaying rates in [42].

The Non-inactivating steady-state K+ current ($I_{KSS}$) is voltage independent ion current, so we describe the current with one activation gating variable as follows.

$$I_{KSS} = G_{KSS}a_{KSS}(V - E_K)$$

$$a_{KSS}(t) = (a_{KSS}(0) - a_{KSS}) e^{-\tau_{KSS}} + a_{KSS}$$

where $G_{Kur}$ and is the maximum whole cell conductance (ms/mf), $a_{KSS}$ is the activation gates. The equations that describe the gating variables are listed in Table I.

The mathematical formulations of steady state activation, steady state inactivation, transition rates, time constants are listed in Table I. We empirically identified the important control variables, and optimally calibrated the control variables to bring the modeled outputs as close as possible to the in-vitro data. The parameters that are closely associated with voltage ($V$), e.g., $x_1$ and $x_3$, were chosen as control variables. Steady state activation and inactivation of $I_{Kslo2}$ and $I_{Kslo}$ channel were adjusted to fit the Boltzmann equation of ST and ST3Gal4-/- cells in in-vitro experiments. Slope of activation/inactivation curve, e.g., $x_2$ and $x_4$ were calibrated to simulate the steady states of the channels. In addition, the time constant $\tau$'s were calibrated to match in-vitro data. The optimal sets of model parameters under both WT and ST3Gal4-/- conditions are given in Section V.

<table>
<thead>
<tr>
<th>TABLE I. MODELS AND CONTROL VARIABLES</th>
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<tbody>
<tr>
<td>Steady States, Transition Rates &amp; Time Constant</td>
</tr>
<tr>
<td>$a_{KSS} = (0.000152\exp(-V+13.5)/7.0)/(0.067083\exp(-V+33.5)/7.0)+1)$</td>
</tr>
</tbody>
</table>

At the cellular level, we modeled the AP of ventricular myocytes with Bondarenko et al [33]. The cellular AP is described by the ordinal differential equation:

$$-C_m \frac{dV}{dt} = I_{Cal} + I_{p(Ca)} + I_{NaCa} + I_{CaB} + I_{Na} + I_{NaB} + I_{NK} + I_{Kto,f} + I_{Kto,s} + I_{K1} + I_{Ks} + I_{Kslow} + I_{KSS} + I_{Kv} + I_{Cl,S} + I_{stim}$$

where $t$ is time, $C_m$ is the cell capacitance, $I_{stim}$ is the external stimulus current which activates the cell from the resting state. The transmembrane currents include the fast Na+ current ($I_{Na}$), the L-type Ca2+ current ($I_{Cal}$), the rapidly recovering transient outward K+ current ($I_{Kto,f}$), the slowly recovering transient outward K+ current ($I_{Kto,s}$), the non-inactivating steady-state voltage-activated K+ current ($I_{Kss}$), the time-independent inwardly rectifying K+ current ($I_{K1}$), the slow delayed rectifier K+ current ($I_{Kv}$), the Na+ /Ca2+ exchange current ($I_{NaCa}$), the Na+ /K+ pump current ($I_{NaK}$), the Ca2+ pump current ($I_{p(Ca)}$), the Ca2+-activated Cl- current, and the background Ca2+ and Na+ currents. Most ionic currents are modeled using Hodgkin-Huxley or Markov-based formulations, including the cell conductance, the gradients of membrane potential and gating dynamics. For details on all the ion-channel kinetics, see Bondarenko et al [33].

IV. MATERIALS AND EXPERIMENTAL DESIGN

As we mentioned above, $K_n$'s share similar gating kinetics and activate at similar range of voltages, it is difficult to separate the K+ currents experimentally with patch clamp protocols. So the currents that pass through all $K_n$ channels were jointly measured and decomposed with an empirical approach. The gating kinetics and individual currents were analyzed and obtained through a bi-exponential decomposition. This section will detail the experimental protocols and the decomposition method used in both in-vitro and in-silico experiments for attaining each K+ current.

4.1 Experimental Protocols

Joint K+ Current ($I_{K,sum}$). The cells were held at the resting potential, -70mV, then depolarized by a series of
adaptive time steps were calculated [41].

The joint K+ current contains six types of currents that flow through various voltage dependent channels, e.g., Kᵩ 4.2, Kᵣ 1.5, Kᵢ 2.1, which contribute differently to the repolarization of the AP in adult mouse ventricular myocytes. As aforementioned, some of these currents, i.e., iK₁, iKₛ and iKᵣ, are significantly small, and have limited contributions to iK,∑. As shown in Figure 3, at the clamp voltage of 30mV, iK₁, iKₛ and iKᵣ are very small, hence their contribution to iK,∑ can be eliminated comparing to iK₀, iK,slow and iK,ss. It may also be noted that the iK,ss does not exhibit any appreciable voltage-dependence. In addition, the iK₀ contributes greatly to the peak of the iK,∑. This gives advantages of separating the kinetics components mathematically by fitting the decaying portion of the joint K+ current with a bi-exponential function [19, 43-46]:

\[ f(t) = A₁ e^{-t/τ₁} + A₂ e^{-t/τ₂} + A₃ \]

where A₁ is the amplitude of the delayed rectifier K+ current (iK,slow) of each current trace, A₂ is the amplitude of the rapidly inactivating transient outward K+ current (iK₀), A₃ is the amplitude of the non-inactivating steady-state K+ current (iK₀). t is the time in ms, and τ₁ and τ₂ are time constants.

![Figure 3. K+ currents at clamp voltage of 30mV](image)

**Steady State Activation.** The amplitudes of each component in each current trace, calculated from the bi-exponential function, were divided by the cell capacitance to generate current density (A/F). The conductance (G) can be obtained through dividing the current by the reverse potential using the following equation:

\[ G = I/(V - Eᵢ) \]

where I is the current at each clamp voltage, V is the clamp voltage, Eᵢ is the reverse potential (82.8mV in our in-vitro experiments). Conductance-voltage relationships were fit with a Boltzmann equation of the form:

\[ G/G_{max} = \frac{1}{1 + \exp(-(V - Vₐ)/Kₐ)} \]

where V is the membrane potentials, Vₐ is the half-activation potential, Kₐ is the slope factor. The normalized data were averaged with those from the other cells to calculate the resulted average G-V curves.

**Steady State Inactivation.** Cells were held at -70 mV and then pre-pulsed to conditioning voltages ranging from -110 mV to 0 mV in 10 mV increments for 10 seconds. Following the conditioning pulses, the cells were depolarized to 30 mV for 4.5 seconds. The magnitude of iK,₀, iK,slow and iK,ss calculated from each current trace were normalized to their corresponding peak currents from the pre-pulse, i.e., the first step to 30mV. The data was fit to a single Boltzmann distribution, from which the mean Vᵢ and Kᵢ values were determined. The fraction of maximal currents is:

\[ I/I_{max} = \frac{1}{1 + \exp((V - Vᵢ)/Kᵢ)} \]

where I/Iₘₐₓ is the ratio of each current to the maximum current, V is the membrane potential, Vᵢ is the voltage of half-inactivation, Kᵢ is the slope factor. In-vitro experiments are collected following the protocols described above, and physical data is from Ednie [41].

**Time constants of Inactivation (tᵢna ct).** In computer experiments, cell were held at -70mV and then stepped to clamp voltage ranging from -50mV to 50mV with 10mV increment. The decaying portion of each current trace is fitted to the exponential equation.

\[ I(t) = \exp(-t/tᵢna ct) \]

where tᵢna ct is the time constant describing the decay rate of each current traces.

### 4.2 Computer Experiments

The computer experiments are aimed at modeling the effects of ST3Gal4⁻/⁻ on each individual K+ currents and further predicting the effects on cardiac electrical signaling of ventricular cells. First, we modified the Kᵩ channel gating equations in the detailed ventricular cell models based on the experimental data in [41]. The current density, steady-state activation and inactivation, inactivation time constant were optimized to fit the experimental results under both ST3Gal4⁻/⁻ and WT conditions. Second, we simulated and compared the variations of each K+ current and the cellular APs with respect to the two conditions, i.e. WT vs ST3Gal4⁻/⁻. dt = 0.01ms was used in model calibrations and in computer experiments at the channel level. Adaptive time steps were used in the experiments at the cellular level, where each cells were stimulated for 40 times to reach stabilities.

Computer models were implemented using the MATLAB R2014a software from MathWorks (Natick, MA, USA) in a Windows 7 (Microsoft, Redmond, WA, USA) 64-bit machine. Computer experiments follow the same pulse protocols as in-vitro experiments to compute the model responses and to calibrate in-silico models. The models of cardiac cells and Kᵩ channels are solved with the use of Ode15s solver at a maximal step size of 1ms.

### V. RESULTS

Table II shows optimal values of control variables for the calibrated models of K+ channels under WT and ST3Gal4⁻/⁻ conditions. Note that the optimal parameters of the WT and ST3Gal4⁻/⁻ models are slightly different in half-activation and half-inactivation voltage as well as slopes. The computer models show best fits to the WT and ST3Gal4⁻/⁻ experimental data with standard pulse protocols (see Section IV).
TABLE II.  OPTIMAL VALUES OF CONTROL VARIABLES FOR WT AND ST3GAL4−/− CELLS

<table>
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<tr>
<th></th>
<th>WT</th>
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</table>

5.1 ST3Gal4−/− shifts SSA to more depolarized potentials

Ultra-rapidly delayed rectifier K⁺ current, I_{Kslow}. As it is mentioned in Section III, we simulated the ultra-rapidly delayed rectifier K⁺ current with two K⁺ currents, i.e., I_{Kslow1} and I_{Kslow2}. The SSA and SSI yields to the optimal fit to the in-vitro data (Figure 4). The modeling results indicated that the SSA of I_{Kslow} channel was shifted to more depolarized potentials along the voltage axis under the ST3Gal4−/− condition, which matches the in-vitro data in [41].

The SSA of I_{Kslow} channel shows a slightly higher inactivation probability when the clamp voltages are greater than -40mV (see Figure 3 b), in spite of the difference are not very significant. The simulated results are consistent with the in-vitro data, i.e., ST3Gal4−/− shifts the SSA linearly in the depolarized direction, and leads to a small delay in the inactivation at higher potentials (> -40mV).

Figure 4. The I_{Kslow} gating under ST3Gal4−/− and WT conditions. (a) Steady-state activation (b) Steady-state inactivation. (In-vitro data as reported by Ednie [41]: WT ● ST3Gal4−/−. In-silico data from the I_{Kslow} model: straight and dashed lines).

To further validate the modeling results, the current density of I_{Kslow} is presented in Figure 5a. The in-silico study shows that ST3Gal4−/− contributes to decreased I_{Kslow} density at smaller, non-saturating membrane potentials (-30mV−20mV).

Transient outward K⁺ current, I_{to}. In addition to I_{Kslow} channel, we also modeled the gating kinetics of I_{to} channel under both ST3Gal4−/− and WT conditions. Figure 6 shows the in-vitro and in-silico SSA and SSI of I_{to} channels. ST3Gal4−/− causes a small rightward shift (~5mV) in SSA, which indicates that the channel opens at higher membrane potentials. However, the SSI curve shows no significant difference under the two conditions. It may be noted that the modeled outputs replicate the physical data well.

The current density of I_{to} was also calculated to evaluate the model accuracy. As shown in Figure 5b, ST3Gal4−/− leads to a reduced density in I_{to} current at more depolarized membrane potentials (>10mV). However, the densities are similar at smaller membrane potentials (-50mV−10mV).

Figure 6 The I_{to} gating under ST3Gal4−/− and WT conditions. (a) Steady-state activation (b) Steady-state inactivation. (In-vitro data as reported by Ednie [41]: WT ● ST3Gal4−/−. In-silico data from the I_{to} model: straight and dashed lines).

Non-inactivating steady-state voltage-activated K⁺ current (I_{Kss}). We also modeled the current-voltage (I-V) relationship of I_{Kss} under both WT and ST3Gal4−/− conditions (see Figure 5c). No significant differences are shown in the I-V curve except ST3Gal4−/− leads to a slightly low magnitudes of I_{Kss} at higher potentials (10mV−50mV).

Figure 5. Current density of I_{Kslow} (a), I_{to} (b) and I_{Kss} (c) under ST3Gal4−/− and WT conditions. (In-vitro data as reported by Ednie [41]: WT ● ST3Gal4−/−. In-silico data from the computer models: straight and dashed lines).
Time constants of inactivation, $\tau_{\text{inact}}$. The time constant of inactivation ($\tau_{\text{inact}}$) were determined by fitting the decaying portion of each current trace to an exponential equation (See Section IV). The $I_{to}$ shows faster decay rates with little difference between WT and ST3Gal4$^{-/}$ conditions. As shown in Figure 7, the $\tau_{\text{inact}}$ for $I_{to}$ at the depolarization of 40mV is 56.69ms and 67.94 ms (62.9±3.6ms and 71.1±4.1ms in in-vitro experiments) in WT and ST3Gal4$^{-/}$ cells respectively. However, comparing to $I_{to}$, the $\tau_{\text{inact}}$ of $I_{k_{\text{slow}}}$ are significantly higher. In addition, the ST3Gal4$^{-/}$ cells show larger inactivation time constants (Figure 7). The $\tau_{\text{inact}}$ for $I_{k_{\text{slow}}}$ at the clamp voltage of 40mV is 1078ms (1089 ±54ms in in-vitro experiments) in WT cells, whereas it is 1185ms (1270 ±55ms in in-vitro experiments) in ST3Gal4$^{-/}$ cells at the same depolarization. The modeled results are closed to the data from in-vitro experiments, which shows the accuracy of the simulation at channel levels.

Recovery from inactivation. We also modeled the recovery from inactivation for the joint K+ current, $I_{k_{\text{sum}}}$ following the standard two-pulse protocol. Cells were held at a membrane potential of -70mV and depolarized to 40mV for 10s. The cells were then returned to -70mV for variant time intervals of 10ms to 5000ms and then depolarized to 40mV for 4.5s. The modeling outcomes are presented in Figure 8, which shows the modeled outcomes are consistent with in-vitro data, i.e., no significant differences are observed between WT and ST3Gal4$^{-/}$ conditions.

5.2 ST3Gal4$^{-/}$ Delays the Repolarization in Ventricular Myocytes

We have also integrated the calibrated models of $K_v$ channels into the ventricular cell model to predict whether and how ST3Gal4 gene deletion influence the electrical activity of cardiac cells. It should be noted that the $K_v$ channels are responsible for multiple phases of the AP. Slightly change in each of these $K_v$ channels may affect the depolarization and repolarization of the ventricular cell, and contribute to cellular excitation. Figure 9 shows the three K+ currents: $I_{k_{\text{slow}}}$, $I_{to}$ and $I_{k_{ss}}$ during a single cardiac cycle and the AP of the ventricular cell. Note that the ST3Gal4$^{-/}$ doesn’t influence the $I_{k_{slow}}$ and $I_{to}$ much comparing to the $I_{k_{ss}}$.

It may be noted that $I_{k_{slow}}$ current shows a smaller peak under ST3Gal4$^{-/}$ condition (Figure 9a). This is caused by the rightward shift in the SSA under the ST3Gal4$^{-/}$ condition (Figure 4a), since the activation of the channels are delayed in ST3Gal4$^{-/}$ cells. Figure 9a also indicates that ST3Gal4$^{-/}$ leads to a slower decay during the 10~20ms, which is likely introduced by a slightly higher inactivation probability at -10mV~40mV (Figure 4b).

Similarly, $I_{to}$ shows a slower decay at 10ms~20ms under the ST3Gal4$^{-/}$ condition (Figure 9b). This can be explained by the rightward shift in SSA (Figure 6a), as well as a slightly delayed inactivation at -20mV~40mV (Figure 6b). However, the $I_{to}$ in the ST3Gal4$^{-/}$ cell shows a higher peak comparing to the WT condition, which may be the cause of the late early repolarization in the AP (Figure 9d).

The contributes of ST3Gal4$^{-/}$ to $I_{k_{ss}}$ are more significant comparing to its impacts on $I_{k_{slow}}$ and $I_{to}$. As shown in Figure 9c, the $I_{k_{ss}}$ presents a lower peak and higher magnitudes in repolarization under the ST3Gal4$^{-/}$ condition. Although the impact of ST3Gal4$^{-/}$ on the I-V of $I_{k_{ss}}$ is limited (Figure 5c), the $I_{k_{ss}}$ is sensitive to smaller changes because it is a non-inactivating current with smaller magnitudes. The smaller peak under the ST3Gal4$^{-/}$ condition is consistent with the results in I-V relationship (Figure 5c), i.e., $I_{k_{ss}}$ shows smaller peak currents at the clamp voltage of 10mV~50mV under the ST3Gal4$^{-/}$ condition. Note that $I_{k_{ss}}$ shows a higher magnitudes during the late repolarization at 15ms~40ms (Figure 9c) under the ST3Gal4$^{-/}$ condition, this is because at the beginning of the late repolarization (6ms~15ms), the AP remains at a higher voltage (Figure 9d) due to the higher peak and the slight delay of inactivation in $I_{to}$ during 10ms~20ms (Figure 9b). The larger AP enables a higher $I_{k_{ss}}$ that is relayed throughout the whole repolarization, and the increased $I_{k_{ss}}$ further leads, jointly with $I_{k_{slow}}$, to a prolonged repolarization.

Figure 9d shows the modeled AP of ventricular cells under both WT and ST3Gal4$^{-/}$ conditions. The cell was stimulated for 40 times, and 30th AP is plotted. It may be noted that depolarization doesn’t show differences because the $K_v$’s are mainly responsible for the repolarization phase of cellular excitation. However, the ST3Gal4$^{-/}$ leads to a significant changes in repolarization. The APDs from the in-vitro experiments at 90% APD (APD$_{90}$) are 20.90ms and 33.00ms.
under the WT and ST3Gal4−/− conditions respectively, and the 75% APD (APD75) are 14.17ms and 24.06ms under the WT and ST3Gal4−/− conditions respectively. This shows reduced sialylation significantly delays the depolarization, and leads to a prolonged ventricular APD. It may be noted that the modeled APDs are slightly smaller than the in-vitro data (WT: APD90: 25.8±3.0ms, APD75: 16.4±1.9ms; ST3Gal4−/−: 36.9±2.3ms, APD75: 21.8±1.5ms) [41]. This is because Bondarenko et al. model uses different data for ionic currents such as \( I_{CaT} \) and \( I_{NaCa} \), therefore, the AP model at the whole-cell level may not perfectly match with the cells that were used in our in-vitro experiments. However, the essential objective of this investigation is to study the effect of reduced sialylation on \( K_v \) channels. So those difference among other channels are not concerned in the current study.

![Graph](image1)

Figure 8 Simulated APs and underlying K+ currents of mouse ventricular myocytes under both ST3Gal4−/− and WT conditions. A: \( I_{CaT} \); B: \( I_{Kto} \); C: AP. Black line: WT condition. Red dash line: ST3Gal4−/− condition.

VI. CONCLUSIONS

Mathematical modeling of cardiac myocytes allows one to investigate the detailed disease mechanisms at molecular levels, i.e. channel level, and predict the effects at other physical scales. We coupled in-silico studies with the wealth of experimental data to model, mechanistically, how reduced glycosylation occurring in the ST3Gal4−/− heart affects \( K_v \) activities and cardiac electrical signaling. The computer experiments showed that ST3Gal4−/− caused decreased current densities among K+ currents, and prolongs the repolarization of the AP in ventricular myocytes. This enriches the fundamental knowledge of how ST3Gal4 defect leads to cardiac dysfunction, and further causes cardiac arrhythmias. Once mechanistic details on how ST3Gal4 gene deletion contributes to aberrant electrical signaling in the heart are better understood, potential therapies can be explored to offset glyco-altered gating in ion channels. Further, new designs of therapies could be suggested to correct aberrant glycosylation, i.e., potential corrective gene, environmental, and/or metabolic therapies that could restore the altered cardiac glycome and resume normal cardiac electrical signaling.

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REFERENCES


**SUPPLEMENTS**

**K+ CURRENT MODELS**

<table>
<thead>
<tr>
<th>List of equations</th>
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<tbody>
<tr>
<td>$i_{to} = G_{Kto}a_{to}i_{to}(V - E_K)$</td>
</tr>
<tr>
<td>$a_{to}(t) = (a_{to}(0) - a_{ss, to}) e^{-\frac{t}{\tau_{to}}} + a_{ss, to}$</td>
</tr>
<tr>
<td>$i_{to}(t) = (i_{to}(0) - i_{ss, to}) e^{-\frac{t}{\tau_{to}}} + i_{ss, to}$</td>
</tr>
<tr>
<td>$a_{to} = (0.000152 \exp(-\frac{(V+13.5)}{7.0}))/\left(0.067083 \exp(-\frac{(V+33.5)}{7.0})+1\right)$</td>
</tr>
<tr>
<td>$\beta_{to} = (0.00095 \exp((V+x_1)/x_2))/\left(0.051335 \exp((V+x_1)/x_2)+1\right)$</td>
</tr>
<tr>
<td>$\tau_{to} = 1/(a_{to} + \beta_{to})$</td>
</tr>
<tr>
<td>$i_{ss, to} = 1/\left(1+\exp(-V/x_1/x_2)\right)$</td>
</tr>
<tr>
<td>$a_{ss} = 0.18064 \exp(x_5(V+x_6))$</td>
</tr>
<tr>
<td>$\beta_{ss} = 0.3956 \exp(x_7(V+x_8))$</td>
</tr>
<tr>
<td>$\tau_{as} = 1/(a_{as} + \beta_{as})$</td>
</tr>
<tr>
<td>$a_{ss, to} = a_{as}(a_{as} + \beta_{as})$</td>
</tr>
</tbody>
</table>

| $i_{Kslow,j} = G_{Kslow, a_{ur,j}}(V - E_g). j = 1, 2$ |
| $a_{ur,j}(t) = (a_{ur,j}(0) - a_{ss,j}) e^{-\frac{t}{\tau_{ur,j}}} + a_{ur,j}$ |
| $i_{ur,j}(t) = (i_{ur,j}(0) - i_{ss,j}) e^{-\frac{t}{\tau_{ur,j}}} + i_{ur,j}$ |
| $a_{ur,j} = 1/\left(1+\exp(-V-x_9/x_10)\right)$ |
| $\beta_{ur,j} = 1/\left(1+\exp(-V-x_9/x_10)\right)$ |
| $\tau_{ur,j} = 1/\left(1+\exp(-V-x_9/x_10)\right)$ |
| $i_{ss,j} = 1/\left(1+\exp(-V-x_9/x_10)\right)$ |
| $a_{as,j} = 0.493 \exp(-0.0629V) + 2.058$ |
| $\beta_{as,j} = 0.493 \exp(-0.0629V) + 2.058$ |

| $i_{Kss} = G_{Kss}a_{Kss}(V - E_g)$ |
| $a_{Kss}(t) = (a_{Kss}(0) - a_{ss,k}) e^{-\frac{t}{\tau_{Kss}}} + a_{ss,k}$ |
| $a_{ks,k} = (1+\exp(-V+x_12/x_13))^{-1} \exp(-0.0862V) + 13.17$ |
| $\beta_{ks,k} = 0.075$ |

**Conductance (mS/µF)**

| $G_{Kto, WT} = 0.203$ |
| $G_{Kto, ST3GAl4+/−} = 0.164$ |
| $G_{Kto, ST3GAl4−/−} = 0.0276$ |
| $G_{Kslow1} = 0.1036$ |
| $G_{Kslow2} = 0.075$ |
| $G_{Kss, WT} = 0.06$ |
| $G_{Kss, ST3GAl4+/−} = 0.06$ |