Effects of Sarcolemmal Background Ca\(^{2+}\) Entry and Sarcoplasmic Ca\(^{2+}\) Leak Currents on Electrophysiology and Ca\(^{2+}\) Transients in Human Ventricular Cardiomyocytes: A Computational Comparison

Molly E. Streiff\(^{1,2}\) and Frank B. Sachse\(^{1,2*}\)

\(^{1}\)Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, United States, \(^{2}\)Department of Biomedical Engineering, University of Utah, Salt Lake City, UT, United States

The intricate regulation of the compartmental Ca\(^{2+}\) concentrations in cardiomyocytes is critical for electrophysiology, excitation-contraction coupling, and other signaling pathways. Research into the complex signaling pathways is motivated by cardiac pathologies including arrhythmia and maladaptive myocyte remodeling, which result from Ca\(^{2+}\) dysregulation. Of interest to this investigation are two types of Ca\(^{2+}\) currents in cardiomyocytes: 1) background Ca\(^{2+}\) entry, i.e., Ca\(^{2+}\) transport across the sarcolemma from the extracellular space into the cytosol, and 2) Ca\(^{2+}\) leak from the sarcoplasmic reticulum (SR) across the SR membrane into the cytosol. Candidates for the ion channels underlying background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak channels include members of the mechano-modulated transient receptor potential (TRP) family. We used a mathematical model of a human ventricular myocyte to analyze the individual contributions of background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak to the modulation of Ca\(^{2+}\) transients and SR Ca\(^{2+}\) load at rest and during action potentials. Background Ca\(^{2+}\) entry exhibited a positive relationship with both [Ca\(^{2+}\)]\(_{i}\) and [Ca\(^{2+}\)]\(_{SR}\). Modulating SR Ca\(^{2+}\) leak had opposite effects of background Ca\(^{2+}\) entry. Effects of SR Ca\(^{2+}\) leak on Ca\(^{2+}\) were particularly pronounced at lower pacing frequency. In contrast to the pronounced effects of background and leak Ca\(^{2+}\) currents on Ca\(^{2+}\) concentrations, the effects on cellular electrophysiology were marginal. Our studies provide quantitative insights into the differential modulation of compartmental Ca\(^{2+}\) concentrations by the background and leak Ca\(^{2+}\) currents. Furthermore, our studies support the hypothesis that TRP channels play a role in strain-modulation of cardiac contractility. In summary, our investigations shed light on the physiological effects of the background and leak Ca\(^{2+}\) currents and their contribution to the development of disease caused by Ca\(^{2+}\) dysregulation.

Keywords: calcium, cardiomyocyte, sarcolemma, sarcoplasmic reticulum, leak
INTRODUCTION

Ca²⁺ concentrations are dynamically controlled in cardiomyocytes by a complex regulatory system comprising ion channels, transporters, exchangers, regulatory proteins, and ion buffers. Intricately regulated levels of Ca²⁺ concentrations are critical for electrical activity, excitation-contraction coupling, and other signaling pathways. In many cardiac diseases, the delicate balance of Ca²⁺ cycling is perturbed. Ca²⁺ dysregulation underlies maladaptive cardiac remodeling. A complete understanding of all components of Ca²⁺ handling is essential for the development of therapeutic strategies to attenuate cardiac pathologies.

Many ion channels underlying the Ca²⁺ signaling in cardiomyocytes are well characterized. Ca²⁺ signaling related to excitation-contraction coupling primarily relies on sarclemmal Ca²⁺ entry through voltage-gated L-type Ca²⁺ channels (LTCC) to trigger Ca²⁺-induced Ca²⁺ release from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR), and, subsequently, Ca²⁺ extrusion via the sodium-calcium exchanger (NCX) and reuptake into the SR through sarco/endoplasmic Ca²⁺-ATPase (SERCA). Other Ca²⁺ currents contribute to the modulation of Ca²⁺ concentrations in cardiomyocytes as well. Of interest to this study are background Ca²⁺ entry through the sarcolemma and SR Ca²⁺ leak. Understanding of the physiological role of these Ca²⁺ currents is still incomplete.

Beyond Ca²⁺ transport across the sarcolemma from the extracellular space into the cytosol through LTCC and NCX, sarclemmal Ca²⁺ transport comprises background Ca²⁺ entry. In resting cardiomyocytes, [Ca²⁺], is of the order of 100 nM, which indicates the existence of a background Ca²⁺ entry pathway to balance the Ca²⁺ efflux of NCX (Eisner et al., 2020). Resting ventricular myocytes depleted of Ca²⁺ stores with caffeine are able to reload the SR by a mechanism that involves extracellular Ca²⁺, demonstrating further evidence of background Ca²⁺ entry (T erracciano and Macleod, 1996). Based on studies measuring background Ca²⁺ influx in rat ventricular myocytes of the order of 2–5 μmol/L per second (Choi et al., 2000) or 4 μmol/L per second (Sankaranarayanan et al., 2017), the background Ca²⁺ entry is approximately 10% of the influx through LTCC current (5–10 μmol/L each action potential) at normal heart rates (Eisner et al., 2020). The identity of background Ca²⁺ flux is still poorly defined, but several channels have been suggested as contributors.

One study identified a Ca²⁺ entry mechanism that is blocked by the nonspecific agent gadolinium (Gd³⁺) (Kupittayanant et al., 2006). Connexin hemichannels are candidates for background Ca²⁺ entry since they can be inhibited by Gd³⁺ (Stout et al., 2002). While connexin hemichannels primarily form pairs to allow ion fluxes between cells at intercalated discs, some are present as hemichannels in the surface membrane of a single cell (Wang et al., 2012; Leybaert et al., 2017) and may, therefore, provide a route for Ca²⁺ entry. However, primary candidates for background Ca²⁺ entry include members of the family of Transient Receptor Potential (TRP) channels, which are also sensitive to Gd³⁺. The mdx mouse model of muscular dystrophy exhibits [Ca²⁺], and [Na⁺], overload that can be blocked by Gd³⁺, and the increase in cation entry has been suggested to involve TRPC channels (Mijares et al., 2014).

Myocytes from old mdx mice exhibit increased expression of a putative stretch-activated channel (SAC), TRPC1. Elevated [Ca²⁺], levels can also be reduced to [Ca²⁺], levels of WT myocytes when exposed to SAC blockers streptomycin or GsMTx-4 (Williams and Allen, 2007; Ward et al., 2008). Upregulated TRPC1 also contributes to increased [Ca²⁺], through SAC in hypertrophic myocardium of rats following isoproterenol injection (Chen et al., 2013).

Background Ca²⁺ entry involved in maladaptive cardiac remodeling was more recently shown to critically depend on both TRPC1 and TRPC4 (Camacho Londono et al., 2015, Camacho Londoño et al., 2021). TRPC6 channels have also been shown to modulate cytosolic Ca²⁺ transients and SR Ca²⁺ load through sarcemomal Ca²⁺ entry (Ahmad et al., 2020). Furthermore, TRPV4 can modulate Ca²⁺ transients and SR load, and participates in hypoosmotic stress-induced cardiomyocyte Ca²⁺ entry (Rubinstein et al., 2014; Jones et al., 2019).

Ca²⁺ transport across the SR membrane from the intracellular store into the cytosol is known as SR Ca²⁺ leak. During an action potential, activation of RyR clusters triggered by Ca²⁺ entry through LTCC results in a synchronized release of a large amount of Ca²⁺ from the SR that forms the Ca²⁺ transient and leads to cardiomyocyte contraction. Activation of RyR clusters causes Ca²⁺ sparks. These sparks are an important pathway for SR Ca²⁺ leak. RyR Ca²⁺ leak can occur also through a mechanism independent of sparks (Santiago et al., 2010). Further, total SR Ca²⁺ leak includes a component separate from RyRs (Zima et al., 2010). Many candidates have been suggested as components of this leak, yet it is still ill-defined. A candidate is the inositol 1,4,5-trisphosphate (IP₃) receptor (IP₃R), which is a Ca²⁺ release channel expressed at lower densities than RyRs in cardiomyocytes and found upregulated in heart failure (HF) (Go et al., 1995; Ai et al., 2005) and therefore may be a relevant contributor to SR Ca²⁺ leak (Zima et al., 2010). Members of the TRP family have also been suggested to contribute to SR Ca²⁺ leak. TRPC1 was found to operate as a SR Ca²⁺ leak channel in skeletal muscle (Berbey et al., 2009) and more recently in cardiomyocytes (Hu et al., 2020). Additional evidence suggests contribution of TRPC6, TRPM8, TRPP2, and TRPV1 to endoplasmic reticulum Ca²⁺ leak in various cell types, although characterization is still incomplete for cardiomyocytes (Lemos et al., 2021).

TRP channels constitute primary candidates for explaining background and leak Ca²⁺ currents through both the sarcolemma and the SR membrane. Interestingly, many members of the TRP family are known to be modulated by stretch (Inoue et al., 2009; Reed et al., 2014; Peyronnet et al., 2016). Cardiomyocyte contractility is known to respond to mechanical stretch in two phases: a rapid and a slow response (Calaghan and White, 1999). The rapid response is the cellular basis for the Frank-Starling Mechanism (FSM). It relies primarily on myofilament overlap and alteration of myofilament Ca²⁺ sensitivity, and does not involve changes in Ca²⁺ transients. The slow response has been termed slow force response (SFR) or stress-induced slow increase
in contractility (SSC), describing the gradual increase in twitch force corresponding to an increase in \([\text{Ca}^{2+}]_i\) transients that develops over several minutes when stretch is sustained. Members of the TRP family are likely candidates for mechanotransduction of the SFR.

Though background and leak \(\text{Ca}^{2+}\) currents are still poorly defined, they may play important roles in regulating \(\text{Ca}^{2+}\) homeostasis and contractility and altered \(\text{Ca}^{2+}\) dynamics in cardiac disease. The lack of complete understanding of the identity and mechanics of these channels, in addition to their relatively small amplitude compared to the voltage-gated \(\text{Ca}^{2+}\) channels, make them difficult to study in vivo. A computational model provides the advantage to study the currents and their roles in isolation from other cellular mechanisms. In this study, we use a mathematical model of a human ventricular myocyte to analyze the individual contributions of background \(\text{Ca}^{2+}\) entry and SR \(\text{Ca}^{2+}\) leak to the modulation of \(\text{Ca}^{2+}\) transients and SR \(\text{Ca}^{2+}\) load. We also assess the effects of the \(\text{Ca}^{2+}\) currents on cellular electrophysiology.

**MATERIALS AND METHODS**

**Mathematical Model of Ventricular Myocyte**

We applied a mathematical model of a human ventricular myocyte (Grandi et al., 2010). The model and subsequent analyses were executed in MATLAB (R2020b). The model includes subsarcolemmal and junctional compartments beyond the cytosol compartment. Total background \(\text{Ca}^{2+}\) current \((I_{\text{Ca,bkg}})\) through the sarcolemma is defined as a summation of junctional \((I_{\text{Ca,bkg,junc}})\) and subsarcolemmal \((I_{\text{Ca,bkg,sl}})\) components:

\[
I_{\text{Ca,bkg}} = I_{\text{Ca,bkg,junc}} + I_{\text{Ca,bkg,sl}}
\]

\[
I_{\text{Ca,bkg,junc}} = F_{\text{junc}} G_{\text{bkg}} (V_m - E_{\text{Ca,junc}}),
\]

\[
I_{\text{Ca,bkg,sl}} = F_{\text{sl}} G_{\text{bkg}} (V_m - E_{\text{Ca,sl}}),
\]

\[
I_{\text{Ca,bkg}} = I_{\text{Ca,bkg,junc}} + I_{\text{Ca,bkg,sl}},
\]

where \(F_{\text{junc}} = 0.11\) and \(F_{\text{sl}} = 0.89\) are constants that determine the fraction of total background current corresponding to the junctional and subsarcolemmal spaces, respectively, \(G_{\text{bkg}}\) is the maximum conductance \((5.513 \times 10^{-4} \text{ A/F})\) of the channels, \(V_m\) is the transmembrane voltage, and \(E_{\text{Ca,junc}}\) and \(E_{\text{Ca,sl}}\) are Nernst potentials corresponding to the junctional and subsarcolemmal spaces, respectively. SR \(\text{Ca}^{2+}\) leak \((J_{\text{leak}})\) describes the \(\text{Ca}^{2+}\) flux out of the SR:

\[
J_{\text{leak}} = K_{\text{leak}} ([\text{Ca}^{2+}]_{\text{SR}} - [\text{Ca}^{2+}]_j),
\]

where \(K_{\text{leak}}\) is the leak constant \((5.348 \times 10^{-6} \text{ ms})\), and \([\text{Ca}^{2+}]_{\text{SR}}\) and \([\text{Ca}^{2+}]_j\) describe the \(\text{Ca}^{2+}\) concentrations in the SR and junctional space, respectively. In this study, we modulated \(G_{\text{bkg}}\) and \(K_{\text{leak}}\) independently and in conjunction to investigate the effects of altered sarcolemmal \(\text{Ca}^{2+}\) entry and altered SR \(\text{Ca}^{2+}\) leak, respectively.

A graphical summary of the \(\text{Ca}^{2+}\) currents in the cell model is shown in Figure 1. The total \(\text{Ca}^{2+}\) current into the junctional \((I_{\text{Ca,tot,junc}})\) and subsarcolemmal \((I_{\text{Ca,tot,sl}})\) compartments are determined by Eqs 5, 6, respectively:

\[
I_{\text{Ca,tot,junc}} = I_{\text{Ca,junc}} + I_{\text{Ca,bkg,junc}} + I_{\text{pCa,junc}} - 2 \cdot I_{\text{NCX,junc}},
\]

\[
I_{\text{Ca,tot,sl}} = I_{\text{Ca,sl}} + I_{\text{Ca,bkg,sl}} + I_{\text{pCa,sl}} - 2 \cdot I_{\text{NCX,sl}},
\]

where \(I_{\text{Ca}}\) describes the L-type \(\text{Ca}^{2+}\) current, \(I_{\text{pCa}}\) describes the sarcolemmal \(\text{Ca}^{2+}\) pump current, and \(I_{\text{NCX}}\) describes the NCX current.
The rate of change of Ca\textsuperscript{2+} concentrations in the junctional compartment [Ca\textsuperscript{2+}]\textsubscript{j}, subsarcolemmal compartment [Ca\textsuperscript{2+}]\textsubscript{sl}, bulk cytosol [Ca\textsuperscript{2+}]\textsubscript{cyt}, and SR [Ca\textsuperscript{2+}]\textsubscript{SR} are given by:

\[
\frac{d[Ca^{2+}]}{dt} = -I_{Ca,B,junc} \cdot \frac{C_{mem}}{V_{junc}} \cdot 2 \cdot F \cdot \frac{J_{Ca,junc}}{V_{junc}} \cdot ([Ca^{2+}]_{sl} - [Ca^{2+}]_{j}) \\
- I_{Ca,B,sl} \cdot \frac{V_{myo}}{V_{junc}} \cdot ( [Ca^{2+}]_{j} - [Ca^{2+}]_{sl} ) - J_{Ca,B,cytosol} \cdot \frac{V_{myo}}{V_{junc}} \cdot ( [Ca^{2+}]_{sl} - [Ca^{2+}]_{cyt} ) \\
+ \frac{J_{Ca,junc}}{V_{junc}} \cdot ([Ca^{2+}]_{j} - [Ca^{2+}]_{sl} ) - J_{Ca,B,sl} \cdot \frac{V_{myo}}{V_{junc}} \cdot ([Ca^{2+}]_{sl} - [Ca^{2+}]_{cyt}) \\
+ \frac{J_{Ca,B,cytosol}}{V_{junc}} \cdot ([Ca^{2+}]_{sl} - [Ca^{2+}]_{cyt}) \\
+ \frac{J_{Ca,sl,myo}}{V_{junc}} \cdot ([Ca^{2+}]_{j} - [Ca^{2+}]_{myo}) - \frac{dC_{sqnb}}{dt} \\
(7)
\]

\[
\frac{d[Ca^{2+}]}{dt} = -I_{SERCA} \cdot \frac{V_{Sr}}{V_{myo}} \cdot ( [Ca^{2+}]_{myo} - [Ca^{2+}]_{Sr} ) - \frac{dC_{sqnb}}{dt} \\
(9)
\]

\[
\frac{d[Ca^{2+}]}{dt} = -I_{Ca,B,sl} \cdot \frac{V_{myo}}{V_{junc}} \cdot ( [Ca^{2+}]_{sl} - [Ca^{2+}]_{myo} ) - \frac{dC_{sqnb}}{dt} \\
(10)
\]

where \(C_{mem}\) is the membrane capacitance, \(F\) is Faraday’s constant, \(V_{junc}\) is the volume of the junctional compartment, \(V_{sl}\) is the volume of the subsarcolemmal compartment, \(V_{myo}\) is the volume of the bulk cytosol, and \(V_{sr}\) is the volume of the SR. \(J_{Ca,junc}\) is the rate of Ca\textsuperscript{2+} flux from junctional to subsarcolemmal compartments and \(J_{Ca,sl,myo}\) is the rate of Ca\textsuperscript{2+} flux from the subsarcolemmal compartment to bulk cytosol. \(J_{Ca,B,cytosol}\) describes the SR Ca\textsuperscript{2+} release and \(J_{SERCA}\) describes the SR Ca\textsuperscript{2+} pump. Ca\textsuperscript{2+} buffering is described by \(I_{Ca,B,junc}\) in the junctional compartment, \(I_{Ca,B,sl}\) in the subsarcolemmal compartment, \(I_{Ca,B,cytosol}\) in the bulk cytosol, and \(dC_{sqnb}/dt\) in the SR. We refer to (Grandi \textit{et al.}, 2010) for more details and the equations describing the mathematical model.

\[d\]

Evaluating the Effects of Background and Leak Ca\textsuperscript{2+} Currents on Ca\textsuperscript{2+} Concentrations

To investigate the effects of altered background Ca\textsuperscript{2+} entry, we modulated \(G_{bkg}\) from 0 to 300\% of its default value, 5.513e-4 A/F. To investigate the effects of altered SR Ca\textsuperscript{2+} leak, we modulated \(K_{leak}\) from 0 to 300\% of its default value, 5.348e-6 ms\textsuperscript{-1}. Simulation durations were 1 min to establish steady state. We analyzed the last beat. We performed sensitivity analyses for the modulation of \(G_{bkg}\) and \(K_{leak}\) independently on measurements of resting \(V_{m}\), maximum \(V_{m}\), action potential duration to 90\% repolarization (APD\textsubscript{90}), diastolic [Ca\textsuperscript{2+}]\textsubscript{i}, systolic [Ca\textsuperscript{2+}]\textsubscript{i}, [Ca\textsuperscript{2+}]\textsubscript{amplitude}, diastolic [Ca\textsuperscript{2+}]\textsubscript{SR}, systolic [Ca\textsuperscript{2+}]\textsubscript{SR}, and [Ca\textsuperscript{2+}]\textsubscript{SR} amplitude. Sensitivity analyses of each measured parameter were then normalized to the measurement of the default model values and fit to the quadratic equation:

\[y(x) = Ax^2 + Bx + C,\]

where \(x\) is the fraction (\%) of default \(G_{bkg}\) or \(K_{leak}\). We evaluated the factor of the quadratic term, \(A\), the linear term, \(B\), and the constant term, \(C\), of this fit for relative comparisons of the effects of modulated background Ca\textsuperscript{2+} current or modulated SR Ca\textsuperscript{2+} leak. This analysis was repeated for the model ran at 1, 2, and 3 Hz electrical excitation.

We subsequently performed a dual-sensitivity analysis for the modulations of \(G_{bkg}\) and \(K_{leak}\) to understand how the two Ca\textsuperscript{2+} currents interact and influence Ca\textsuperscript{2+} handling in the cardiomyocyte together.

RESULTS

Qualitative Changes in \(V_{m}\), [Ca\textsuperscript{2+}]\textsubscript{i}, and [Ca\textsuperscript{2+}]\textsubscript{SR} When Background Ca\textsuperscript{2+} Entry is Modulated

We first examined the changes in \(V_{m}\), [Ca\textsuperscript{2+}]\textsubscript{i}, and [Ca\textsuperscript{2+}]\textsubscript{SR} following modulation of the background Ca\textsuperscript{2+} entry current, \(I_{Ca,bk}\) at pacing rates of 1, 2, and 3 Hz (Figure 2). Changes to features of the action potential were minimal (Figure 2A). Resting \(V_{m}\) exhibited a positive relationship with \% \(G_{bkg}\) (Figure 2B), while maximum depolarization exhibited a negative relationship (Figure 2C) with increasing background Ca\textsuperscript{2+} current. APD\textsubscript{90} showed a positive relationship, where increased background Ca\textsuperscript{2+} entry lengthens the time for repolarization (Figure 2D). We measured diastolic and systolic values, as well as amplitude of [Ca\textsuperscript{2+}]\textsubscript{i}, transients (Figure 2E). All measurements demonstrate positive relationships with increasing background Ca\textsuperscript{2+} entry; the increase in systolic [Ca\textsuperscript{2+}]\textsubscript{i}, (Figure 2F) is greater than the increase in diastolic [Ca\textsuperscript{2+}]\textsubscript{i}, (Figure 2G), especially at the slowest pacing rate, so the amplitude increases a considerable amount (Figure 2H). We measured the same parameters of [Ca\textsuperscript{2+}]\textsubscript{SR} transients, which also demonstrate an increase in amplitude caused by increased background Ca\textsuperscript{2+} entry (Figures 2I,J). The release was initiated from increased diastolic [Ca\textsuperscript{2+}]\textsubscript{SR} (Figure 2J) and ended in reduced systolic [Ca\textsuperscript{2+}]\textsubscript{SR} (Figure 2K). The relationships between background Ca\textsuperscript{2+} entry and features of the [Ca\textsuperscript{2+}]\textsubscript{SR} transient were also augmented at the slowest pacing rate.

Qualitative Changes in \(V_{m}\), [Ca\textsuperscript{2+}]\textsubscript{i}, and [Ca\textsuperscript{2+}]\textsubscript{SR} When SR Ca\textsuperscript{2+} Leak is Modulated

Changes following independent modulation of the SR Ca\textsuperscript{2+} leak current, \(I_{leak}\), were also examined (Figure 3). The effects of modulating \(K_{leak}\) on \(V_{m}\) were small (Figure 3A). Resting \(V_{m}\) exhibited a slight positive relationship (Figure 3B). Maximum \(V_{m}\) increased with increasing \(K_{leak}\) at 1 Hz pacing but decreased with \(K_{leak}\) at 2 and 3 Hz pacing (Figure 3C). Repolarization time measured as APD\textsubscript{90} was unaltered by modulations of \(K_{leak}\) (Figure 3D). Diastolic [Ca\textsuperscript{2+}]\textsubscript{i}, was also relatively unaffected by modulations of \(K_{leak}\) (Figures 3E,F). However systolic [Ca\textsuperscript{2+}]\textsubscript{i}, and thus also [Ca\textsuperscript{2+}]\textsubscript{i}, amplitude decreased with increasing \(K_{leak}\) (Figures 3G,H). The effect was strongest at the slowest pacing frequency of 1 Hz. Alterations in [Ca\textsuperscript{2+}]\textsubscript{SR} were also strongest at 1 Hz pacing. Diastolic [Ca\textsuperscript{2+}]\textsubscript{SR} exhibited a negative relationship with \(K_{leak}\) (Figure 3I), while systolic [Ca\textsuperscript{2+}]\textsubscript{SR} exhibits a positive relationship.

\[d\]
FIGURE 2 | Effects of modulating the background Ca\textsuperscript{2+} entry current, \(I_{\text{Ca,bk}}\), on \(V_m\), [Ca\textsuperscript{2+}]\(_i\), and [Ca\textsuperscript{2+}]\(_{SR}\). (A) Example action potentials for 50% and 200% \(G_{\text{Ca,bk}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(G_{\text{Ca,bk}}\) on measurements of (B) resting \(V_m\), (C) maximum \(V_m\), and (D) time to 90% repolarization at 1, 2 and 3 Hz pacing. (E) Example [Ca\textsuperscript{2+}]\(_i\) transients for 50% and 200% \(G_{\text{Ca,bk}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(G_{\text{Ca,bk}}\) on measurements of (F) diastolic [Ca\textsuperscript{2+}]\(_i\), (G) systolic [Ca\textsuperscript{2+}]\(_i\), and (H) [Ca\textsuperscript{2+}]\(_i\) amplitude at 1, 2 and 3 Hz pacing. (I) Example [Ca\textsuperscript{2+}]\(_{SR}\) transients for 50% and 200% \(G_{\text{Ca,bk}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(G_{\text{Ca,bk}}\) on measurements of (J) diastolic [Ca\textsuperscript{2+}]\(_{SR}\), (K) systolic [Ca\textsuperscript{2+}]\(_{SR}\), and (L) [Ca\textsuperscript{2+}]\(_{SR}\) amplitude at 1, 2 and 3 Hz pacing.

FIGURE 3 | Effects of modulating the SR Ca\textsuperscript{2+} leak flux, \(J_{\text{leak}}\), on \(V_m\), [Ca\textsuperscript{2+}]\(_i\), and [Ca\textsuperscript{2+}]\(_{SR}\). (A) Example action potentials for 50% and 200% \(K_{\text{leak}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(K_{\text{leak}}\) on measurements of (B) resting \(V_m\), (C) maximum \(V_m\), and (D) time to 90% repolarization at 1, 2 and 3 Hz pacing. (E) Example [Ca\textsuperscript{2+}]\(_i\) transients for 50% and 200% \(K_{\text{leak}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(K_{\text{leak}}\) on measurements of (F) diastolic [Ca\textsuperscript{2+}]\(_i\), (G) systolic [Ca\textsuperscript{2+}]\(_i\), and (H) [Ca\textsuperscript{2+}]\(_i\) amplitude at 1, 2 and 3 Hz pacing. (I) Example [Ca\textsuperscript{2+}]\(_{SR}\) transients for 50% and 200% \(K_{\text{leak}}\) at 1 Hz pacing. Sensitivity analyses of modulating \(K_{\text{leak}}\) on measurements of (J) diastolic [Ca\textsuperscript{2+}]\(_{SR}\), (K) systolic [Ca\textsuperscript{2+}]\(_{SR}\), and (L) [Ca\textsuperscript{2+}]\(_{SR}\) amplitude at 1, 2 and 3 Hz pacing.
relationship with $K_{\text{leak}}$ (Figure 3K), both contributing to reduced \([Ca^{2+}]_{\text{SR}}\) amplitude with increased $K_{\text{leak}}$ (Figure 3L).

**Summary and Comparison of Independent Modulation of Background and Leak Currents**

For comparison of the effects of altered $G_{\text{bkg}}$ or $K_{\text{leak}}$ on features of the action potential, we normalized the measurements of resting $V_m$, maximum $V_m$, and APD$_{90}$ to the default measurements from the model for the given pacing frequency (Figures 4A–F). The relationships of measured vs. modified parameter, both represented as fraction (%) vs. default values, were fit to a 2nd order polynomial model for quantification of the effects (Figures 4G–I). The quadratic term of the fit is negligible for resting $V_m$ and maximum $V_m$, demonstrating that these measurements exhibit primarily linear relationships with $G_{\text{bkg}}$ and $K_{\text{leak}}$ (Figure 4G). However, the polynomial fit of APD$_{90}$ to $G_{\text{bkg}}$ has a strong quadratic term, demonstrating a non-linear relationship between APD$_{90}$ and $G_{\text{bkg}}$ (Figure 4G). Since the relationships of resting $V_m$ and maximum $V_m$ are primarily linear, the linear term of the quadratic polynomial fit demonstrates the sensitivity of the measurements to changes in $G_{\text{bkg}}$ or $K_{\text{leak}}$ (Figure 4H). Both $G_{\text{bkg}}$ and $K_{\text{leak}}$ modulation have a positive relationship with resting $V_m$, but the changes are negligible for $K_{\text{leak}}$ and minimal for $G_{\text{bkg}}$, never exceeding an increase greater than 2% of the default model’s resting $V_m$.

Maximum $V_m$ had a strong negative linear relationship with $G_{\text{bkg}}$ for all pacing frequencies, especially at 1 Hz pacing. Maximum $V_m$ had a positive linear relationship with $K_{\text{leak}}$ at 1 Hz pacing but negatives linear relationship for 2 and 3 Hz. The constant of the quadratic polynomial model represents the measurements for the modulated currents set to 0 (Figure 4I). These results revealed that resting $V_m$ was largely unchanged by pacing rate. Maximum $V_m$ was slightly increased with no $I_{\text{Cabk}}$ and slightly reduced with no $I_{\text{leak}}$ at 1 Hz and slightly increased with no $I_{\text{leak}}$ at 3 Hz. APD$_{90}$ decreased without $I_{\text{Cabk}}$ but remained unchanged without $K_{\text{leak}}$.

Figure 5 contains a summary of \([Ca^{2+}]_i\), normalized by measurements of the default model for the sensitivity
analyses of $G_{bkg}$ and $K_{leak}$ modulation. The relationships of measured parameter vs. modified parameter, both represented as % default values, were fit to a 2nd order polynomial model for quantification of the effects. The quadratic term indicates nonlinearity of the relationship (Figure 5G). The quadratic term of the diastolic $[Ca^{2+}]_i$ fits were marginal, indicating a primarily linear relationship. For both $G_{bkg}$ and $K_{leak}$ modulations, we noticed a nonlinearity associated with systolic $[Ca^{2+}]_i$ and consequently the $[Ca^{2+}]_i$ amplitude, with the greatest degree of nonlinearity associated with the lowest pacing frequency. The linear term of the quadratic fit showed a strong linear sensitivity of the measured parameter to changes in $G_{bkg}$ or $K_{leak}$ (Figure 5H). The positive sign of this term for $G_{bkg}$ modulations for each measurement, diastolic, systolic, and amplitude, demonstrates the positive relationship of these parameters with $G_{bkg}$, with greater slope of the relationship for systolic and amplitude measurements. The linear term for diastolic $[Ca^{2+}]_i$ sensitivity to $K_{leak}$ was negative but very small, demonstrating that diastolic $[Ca^{2+}]_i$ exhibits negligible sensitivity to SR Ca$^{2+}$ leak. The relationship of systolic $[Ca^{2+}]_i$ sensitivity to $K_{leak}$ was negative, with the largest value for 1 Hz pacing. The same is true for $[Ca^{2+}]_i$, transient amplitude. The constant term of the quadratic polynomial fits corresponds to the % default if the modulated channel were 0 (Figure 5I). Elimination of $I_{Cabk}$ resulted in a reduction of diastolic $[Ca^{2+}]_i$, systolic $[Ca^{2+}]_i$, and $[Ca^{2+}]_i$ amplitude, with the greatest reductions at 1 Hz pacing. Elimination of $I_{leak}$ did not affect diastolic $[Ca^{2+}]_i$, but results in increased systolic $[Ca^{2+}]_i$, and $[Ca^{2+}]_i$ amplitude, especially at 1 Hz pacing.

Measurements of diastolic and systolic $[Ca^{2+}]_{SR}$ and $[Ca^{2+}]_{SR}$ transient amplitude, normalized by measurements of the default model, for the sensitivity analyses of $G_{bkg}$ and $K_{leak}$ modulations are summarized in Figure 6. Again, a 2nd order polynomial model for the normalized sensitivity analyses provided information about the relationships. The quadratic term of the fits demonstrates that systolic $[Ca^{2+}]_{SR}$ and $[Ca^{2+}]_{SR}$ transient amplitude both exhibited nonlinearity in the relationship to
modulated G_{bkg} (Figure 6G). The linear term of the relationships demonstrated the best representation sensitivity of the measurements to G_{bkg} or K_{leak} modulation (Figure 6H). The sign of this term was opposite for each measured parameter for G_{bkg} vs. K_{leak} modulation but similar in amplitude. While the changes to [Ca^{2+}]_{SR} transients all contributed to a positive correlation between G_{bkg} and [Ca^{2+}]_{SR} transient amplitude, the opposite changes all contribute to a negative correlation between K_{leak} and [Ca^{2+}]_{SR} transient amplitude. The constant term of the polynomial model, which corresponds to the % of default model measurements if the current is set to 0, showed that in the absence of I_{Ca_{bkg}}, diastolic [Ca^{2+}]_{SR} is lower, systolic [Ca^{2+}]_{SR} is higher, and [Ca^{2+}]_{SR} amplitude is reduced (Figure 6I). In the absence of I_{Ca_{leak}}, diastolic [Ca^{2+}]_{SR} was slightly elevated, systolic [Ca^{2+}]_{SR} was reduced, and the amplitude of [Ca^{2+}]_{SR} was greater. The effects on all measurements of [Ca^{2+}]_{SR} following modulation of G_{bkg} or K_{leak} were stronger at 1 Hz pacing than at faster pacing frequencies.

FIGURE 6 | Summary of effects of modulating G_{bkg} or K_{leak} independently on [Ca^{2+}]_{SR}. Sensitivity analyses of modulating G_{bkg} on measurements of (A) diastolic [Ca^{2+}]_{SR}, (B) systolic [Ca^{2+}]_{SR}, and (C) [Ca^{2+}]_{SR} amplitude normalized by the default model parameters. Sensitivity analyses of modulating K_{leak} on measurements of (D) diastolic [Ca^{2+}]_{SR}, (E) systolic [Ca^{2+}]_{SR}, and (F) [Ca^{2+}]_{SR} amplitude normalized by the default model parameters. Summary of the (G) quadratic term, (H) linear term and (I) constant term calculated by quadratic polynomial fits the normalized sensitivity analyses in (A–H).

Effects of Dual Modulation of Background Ca^{2+} Entry and SR Ca^{2+} Leak on Vm, [Ca^{2+}]_i, and [Ca^{2+}]_SR
With a thorough understanding of how G_{bkg} and K_{leak} modulations independently affect features of the action potential, [Ca^{2+}]_i and [Ca^{2+}]_SR transients, we subsequently performed a dual-parameter sensitivity analysis of G_{bkg} and K_{leak} together for 1 Hz pacing (Figure 7). Resting V_m is affected minimally by G_{bkg} and negligibly by K_{leak}, apparent by the nearly horizontal contour lines (Figure 7A). Both increasing G_{bkg} and increasing K_{leak} resulted in increased V_m, but increasing G_{bkg} makes a larger contribution. Maximum V_m was modulated in opposite directions by G_{bkg} and K_{leak} (Figure 7B). Increasing G_{bkg} reduces maximum V_m while increasing K_{leak} increased maximum V_m, but G_{bkg} is the slightly more dominant effect. APD_{90} experienced the greatest increase at large increases in G_{bkg} and small K_{leak} (Figure 7C).
Diastolic $[\text{Ca}^{2+}]_i$ was primarily affected by a positive relationship to $G_{\text{bgk}}$, while $K_{\text{leak}}$ appeared to make no contribution (Figure 7D). Interestingly, for all other $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{SR}}$ measurements, the opposing effects of $G_{\text{bgk}}$ and $K_{\text{leak}}$ appeared to balance with a very similar linear relationship (Figures 7E–I). The contour lines of the dual-parameter sensitivity analyses for systolic $[\text{Ca}^{2+}]_i$, $[\text{Ca}^{2+}]_{\text{SR}}$, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$, and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

If both background and leak currents were modulated along this relationship, measured parameters remained relatively unchanged and only diastolic $[\text{Ca}^{2+}]_i$ increased. An example of balanced background $[\text{Ca}^{2+}]_i$ and increased $[\text{Ca}^{2+}]_{\text{SR}}$, amplitude, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

If both background and leak currents were modulated along this relationship, measured parameters remained relatively unchanged and only diastolic $[\text{Ca}^{2+}]_i$, increased. An example of balanced background $[\text{Ca}^{2+}]_i$ and systolic $[\text{Ca}^{2+}]_i$, increased. An example of balanced background $[\text{Ca}^{2+}]_i$, and increased $[\text{Ca}^{2+}]_{\text{SR}}$, amplitude, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

If both background and leak currents were modulated along this relationship, measured parameters remained relatively unchanged and only diastolic $[\text{Ca}^{2+}]_i$, increased. An example of balanced background $[\text{Ca}^{2+}]_i$, and increased $[\text{Ca}^{2+}]_{\text{SR}}$, amplitude, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

If both background and leak currents were modulated along this relationship, measured parameters remained relatively unchanged and only diastolic $[\text{Ca}^{2+}]_i$, increased. An example of balanced background $[\text{Ca}^{2+}]_i$, and increased $[\text{Ca}^{2+}]_{\text{SR}}$, amplitude, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

If both background and leak currents were modulated along this relationship, measured parameters remained relatively unchanged and only diastolic $[\text{Ca}^{2+}]_i$, increased. An example of balanced background $[\text{Ca}^{2+}]_i$, and increased $[\text{Ca}^{2+}]_{\text{SR}}$, amplitude, diastolic and systolic $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude all exhibited strikingly similar slopes.

DISCUSSION

In this study, we evaluated the contributions of background $[\text{Ca}^{2+}]_i$ entry and SR $[\text{Ca}^{2+}]_{\text{SR}}$ leak to $V_m$ and $[\text{Ca}^{2+}]_i$ concentrations in the SR and cytosol in cardiomyocytes in silico. Our investigations shed light on the differential effects of background and leak $[\text{Ca}^{2+}]$ currents in physiology, and also provide insight into their contributions to disease development due to $[\text{Ca}^{2+}]$ dysfunction. Below, we discussed background $[\text{Ca}^{2+}]_i$ entry as a mechanism to positively modulate $[\text{Ca}^{2+}]_i$ and SR $[\text{Ca}^{2+}]_{\text{SR}}$ as a critical balancing mechanism to maintain homeostasis.

Background $[\text{Ca}^{2+}]_i$ Entry Positively Modulates $[\text{Ca}^{2+}]$ Concentrations

Our results show that background $[\text{Ca}^{2+}]_i$ entry has a positive relationship with diastolic $[\text{Ca}^{2+}]_i$, and $[\text{Ca}^{2+}]_{\text{SR}}$, and the amplitude of their transients (Figure 2). The small increase in diastolic $[\text{Ca}^{2+}]_i$, is the direct result of an increase in background $[\text{Ca}^{2+}]_i$ entry, and the increase in $[\text{Ca}^{2+}]_{\text{SR}}$ follows as SERCA responds to pump the extra $[\text{Ca}^{2+}]$ into the SR. The small increase in diastolic $[\text{Ca}^{2+}]_i$ amplifies $[\text{Ca}^{2+}]_i$-induced-$[\text{Ca}^{2+}]$ release through RyRs, explaining the increased transient amplitudes. This supports the physiological role of background $[\text{Ca}^{2+}]_i$ entry in increasing $[\text{Ca}^{2+}]$ concentrations. These results replicate prior findings for TRP family members suggested as $[\text{Ca}^{2+}]_i$ entry channels. For example, we provided evidence for TRPC6 as background $[\text{Ca}^{2+}]_i$ entry in neonatal rat ventricular

![Figure 7](https://example.com/figure7.png)

**Figure 7** Dual-parameter sensitivity analysis of modulating $G_{\text{bgk}}$ and $K_{\text{leak}}$ at 1 Hz pacing. Surface plots with contour lines for measurements of (A) resting $V_m$, (B) maximum $V_m$, (C) APD$_{90}$, (D) diastolic $[\text{Ca}^{2+}]_i$, (E) systolic $[\text{Ca}^{2+}]_i$, (F) $[\text{Ca}^{2+}]_i$ amplitude, (G) diastolic $[\text{Ca}^{2+}]_{\text{SR}}$, (H) systolic $[\text{Ca}^{2+}]_{\text{SR}}$, and (I) $[\text{Ca}^{2+}]_{\text{SR}}$ amplitude.
myocytes (Ahmad et al., 2020). Overexpression of TRPC6 contributes to both elevated \([\text{Ca}^{2+}]_i\) and \([\text{Ca}^{2+}]_{\text{SR}}\). TRPC3 and TRPC6 have also both been implicated in the stress-induced slow increase in \([\text{Ca}^{2+}]_i\) and increased \([\text{Ca}^{2+}]_i\) transients contributing to the SFR (Seo et al., 2014; Yamaguchi et al., 2017; Yamaguchi et al., 2018). These studies highlight one potential role for \(\text{Ca}^{2+}\) entry in strained myocytes. Many of the candidates suggested as background \(\text{Ca}^{2+}\) entry channels are known to be modulated by stretch (Inoue et al., 2009; Reed et al., 2014; Peyronnet et al., 2016), so strained myocytes would exhibit an increase in background \(\text{Ca}^{2+}\) entry, leading to elevated diastolic levels and larger transients. This mechanism likely contributes to the SFR, increased contractile force following sustained stretch.

SR \(\text{Ca}^{2+}\) Leak Negatively Modulates \(\text{Ca}^{2+}\) Concentrations as a Balancing Mechanism

SR \(\text{Ca}^{2+}\) leak had only a marginal effect on diastolic \([\text{Ca}^{2+}]_i\), but reduced \([\text{Ca}^{2+}]_{\text{SR}}\) and the amplitude of \(\text{Ca}^{2+}\) transients (Figure 3). In general, modulating SR \(\text{Ca}^{2+}\) leak had the opposite effects of background \(\text{Ca}^{2+}\) entry, except for the weak effect on diastolic \([\text{Ca}^{2+}]_i\). The leaked \(\text{Ca}^{2+}\) is removed from the cytosol effectively by NCX, which is why it does not affect free cytosolic levels but reduces SR \(\text{Ca}^{2+}\). On its own, this complicates the understanding of the physiological role of SR \(\text{Ca}^{2+}\) leak and the purpose of reduced SR load. When considering the dual-parameter sensitivity analysis, it became however evident that while background \(\text{Ca}^{2+}\) entry responds to increased needs with increased \(\text{Ca}^{2+}\), SR \(\text{Ca}^{2+}\) leak likely functions as a critical balancing component to regulate SR stores and maintain \(\text{Ca}^{2+}\) homeostasis. The combined effects of increasing both background \(\text{Ca}^{2+}\) entry and SR \(\text{Ca}^{2+}\) leak exhibit a linear relationship, represented by the contour lines of the dual sensitivity plot for systolic \([\text{Ca}^{2+}]_i\), [\(\text{Ca}^{2+}\)], amplitude, diastolic and systolic \([\text{Ca}^{2+}]_{\text{SR}}\) and \([\text{Ca}^{2+}]_{\text{SR}}\) amplitude (Figure 7). If both background and leak currents are modulated along this relationship, measured parameters remain relatively unchanged and only diastolic \([\text{Ca}^{2+}]_i\) increases. Examples of balanced background \(\text{Ca}^{2+}\) entry and SR \(\text{Ca}^{2+}\) leak in Figure 8 demonstrate that modulations in \(G_{\text{big}}\) and \(K_{\text{leak}}\) can be balanced in a way to cancel out the large opposing effects they have on \(\text{Ca}^{2+}\) transient amplitudes (Figure 8). This balancing mechanism of SR \(\text{Ca}^{2+}\) leak could be critical to prevent \(\text{Ca}^{2+}\) overload in the cell. Leak in the form of RyR sparks has been demonstrated as SR load regulator to prevent overload, with a steep dependency on \([\text{Ca}^{2+}]_{\text{SR}}\) (Shannon et al., 2002). However, large \(\text{Ca}^{2+}\) release events through RyR sparks increase sensitivity for arrhythmia (George, 2008). RyR sparks are most prevalent at high \([\text{Ca}^{2+}]_{\text{SR}}\), but non-spark RyR leak and non-RyR leak do not appear to exhibit the same steep dependency on \([\text{Ca}^{2+}]_{\text{SR}}\) and therefore might function differently. Thus, a mechanism of non-RyR leak may be to regulate compartmental \(\text{Ca}^{2+}\) before the cells become overloaded, and RyR sparks increase as a more extreme measure.

Like channels involved in background \(\text{Ca}^{2+}\) entry in cardiomyocytes, candidates for SR \(\text{Ca}^{2+}\) leak also include members of the TRP family and were suggested to be mechano-modulated (Inoue et al., 2009; Reed et al., 2014; Peyronnet et al., 2016). This indicates that the background and leak \(\text{Ca}^{2+}\) currents could be modulated in conjunction. Non-RyR leak that can be modulated by stretch may provide a more moderate and steady...
regulation on a beat-by-beat basis in conjunction with background Ca\(^{2+}\) entry modulated by stretch. Recently we demonstrated that TRPC1 constitutes an SR Ca\(^{2+}\) leak channel, and its overexpression resulted in decreased SR Ca\(^{2+}\) load (Hu et al., 2020). TRPC1 channels are suggested to be modulated by stretch, indicating that the reduction in SR Ca\(^{2+}\) load could be a regulatory mechanism to match increased background Ca\(^{2+}\) entry through, e.g., TRPC6 channels. We speculate that background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak fulfill a critical homeostatic function in the modulation of Ca\(^{2+}\) concentrations throughout the cardiomyocyte in response to strain.

Background and Leak Ca\(^{2+}\) Currents May Contribute to Hypertrophy and HF Under Chronic Pressure Overload

Both background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak through TRP channels are likely to be modulated by cardiomyocyte strain (Inoue et al., 2009; Reed et al., 2014; Peyronnet et al., 2016). Under chronic pressure overload conditions, strain-modulation of TRPC channels could increase background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak, and thus dysregulate Ca\(^{2+}\). Cardiac disease is perpetuated by Ca\(^{2+}\) dysregulation, and a stray from its homeostatic balance. Some of the suggested ion channels for these Ca\(^{2+}\) currents were found to be upregulated in models of cardiac disease, suggesting a role in pathogenesis (Ahmad et al., 2017; Hof et al., 2019). Diastolic [Ca\(^{2+}\)], is elevated in HF causing diastolic dysfunction (Eisner et al., 2020). Elevated background Ca\(^{2+}\) entry could be a contributing factor. Two different models of HF with preserved ejection fraction (HFpEF) display increases in both diastolic and systolic [Ca\(^{2+}\)], (Curl et al., 2018; Rouhana et al., 2019). A hypothesis is that a major difference in Ca\(^{2+}\) handling between HFrEF and HF with reduced ejection fraction (HFrEF) is preserved [Ca\(^{2+}\)]SR in HFrEF vs. reduced [Ca\(^{2+}\)]SR in HFrEF (Eisner et al., 2020). The decreased SR Ca\(^{2+}\) content contributes largely to the decrease in systolic [Ca\(^{2+}\)], and contractile dysfunction (Bers, 2006). Based on the demonstration of a balancing mechanism between background Ca\(^{2+}\) entry and SR Ca\(^{2+}\) leak in this study, it is reasonable to speculate a difference between maintenance of this balance in HFrEF vs. a stray from this balanced relationship towards overcompensated leak in HFrEF. In addition to reducing SR Ca\(^{2+}\) available for release, causing systolic dysfunction, increased SR Ca\(^{2+}\) leak can be problematic, e.g., triggering arrhythmias and being energetically costly due to increased use of ATP to repump Ca\(^{2+}\) (Bers, 2014). Understanding the balance of background and leak Ca\(^{2+}\) currents in cardiomyocytes and how they affect Ca\(^{2+}\) homeostasis and remodeling in disease will be critical to develop effective drug therapies targeting Ca\(^{2+}\) channels.

Modulation of ICabk and Jleak has Marginal Effects on Action Potentials

Modulating K\(_{\text{leak}}\) had negligible effects on the action potential for any pacing frequency (Figure 4). For the values of K\(_{\text{leak}}\) tested, we found that the SR Ca\(^{2+}\) leak flux does not significantly contribute to sarcolemmal electrophysiology. Modulating G\(_{\text{bkg}}\) has marginal effects on features of action potentials (Figure 4). While G\(_{\text{bkg}}\) positively correlates with increased resting V\(_{\text{m}}\), an increase to 300% G\(_{\text{bkg}}\) only resulted in <2% change from basal resting V\(_{\text{m}}\). This minimal change in resting potential is unlikely to be functionally relevant. An increase to 300% G\(_{\text{bkg}}\) also reduces maximum depolarization by 7% for 1 Hz pacing. The largest effect is an increase in action potential duration (APD90) by around 10% for maximal G\(_{\text{bkg}}\) modulation. It has also been shown that APD prolongation leads to increased Ca\(^{2+}\) (Bouchard et al., 1995), suggesting a positive feedback loop for electrical and Ca\(^{2+}\) signaling. Another important note is that APD increase is known to be inotropic, e.g., in rat ventricular myocytes (Bouchard et al., 1995). This indicates another mechanism for the contribution of background Ca\(^{2+}\) entry to contractility. Conversely, prolonged APD can induce torsades de pointes tachycardia, leading to life-threatening ventricular fibrillation (Roden and Hoffman, 1985; Ravens and Cerbai, 2008).

Limitations

Mathematical modeling of cellular electrophysiology provides a valuable resource for studying how aspects of cellular physiology interact and affect one another. It provides a means to investigate questions that cannot be easily answered in vivo. However, there are also caveats of mathematical modeling that should be considered. It should be noted that the definitions of ICabk and Jleak used in this model are general simplifications and meant to reproduce poorly defined currents. The equations lack specific gating conditions of the currents. The current equations were not parameterized to match experimental data which is only incompletely characterized in human ventricular myocytes. Instead, the current equations are adjusted such that the model reproduces overall physiological action potentials and calcium transients. This is an important consideration, since the magnitudes of these currents could be largely different in living cells. Thus, interpreting the results of this study should focus on the qualitative trends. As the specific ion channels that contribute to Ca\(^{2+}\) entry and leak are identified and characterized, future work can aim to refine the current definitions and provide...
detailed current models to replace the general simplifications of ICabk and Jleak.

In a similar way, other ion currents in the model are not fully defined. For example, some K+ channels and isoforms of the Na+/K+-ATPase are modulated by localized Ca2+ concentrations (Tian and Xie, 2008; Weisbrod, 2020). However, the model does not include any Ca2+-dependent terms in the definitions of these currents. The inclusion of these interactions may alter the effects we see on Vm in this study. Future work could address this limitation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

REFERENCES

Ahmad, A. A., Streiff, M., Hunter, C., Hu, Q., and Sachse, F. B. (2017). Physiological and Pathophysiological Role of Transient Receptor Potential Canonical Channels in Cardiac Myocytes. Prog. Biophys Mol. Biol. 130, 254–263. doi:10.1016/j.pbiomolbio.2017.06.005

Ahmad, A. A., Streiff, M. E., Hunter, C., and Sachse, F. B. (2020). Modulation of Calcium Transients in Cardiomyocytes by Transient Receptor Potential Canonical 6 Channels. Front. Physiol. 11, 44. doi:10.3389/fphys.2020.00044

Ai, X., Curran, J. W., Shannon, T. R., Bers, D. M., and Pogwizd, S. M. (2005). Ca 2+/K+-ATPase are modulated by localized Ca2+ concentrations (Tian and Xie, 2008; Weisbrod, 2020). However, the model does not include any Ca2+-dependent terms in the definitions of these currents. The inclusion of these interactions may alter the effects we see on Vm in this study. Future work could address this limitation.

AUTHOR CONTRIBUTIONS

MS and FS designed the study. MS implemented the modeling, analyzed simulation data and drafted the manuscript. All authors critically revised the manuscript and approved the version to be published.

FUNDING

This work was supported by Nora Eccles Treadwell Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.916278/full#supplementary-material
Jones, J. L., Peana, D., Veteto, A. B., Lambert, M. D., Nourian, Z., Karasseva, N. G., et al. (2019). TRPV4 Increases Cardiomyocyte Calcium Cycling and Contractility yet Contributes to Damage in the Aged Heart Following Hypoosmotic Stress. *Cardiovasc. Res.* 115, 46–56. doi:10.1093/cvr/cvy156

Kupittayanant, P., Trafford, A. W., Diaz, M. E., and Eisner, D. A. (2006). A Mechanism Distinct from the L-type Ca Current or Na-Ca Exchange Contributes to Ca Entry in Rat Ventricular Myocytes. *Cell Calcium* 39, 417–423. doi:10.1016/j.ceca.2006.01.011

Lemos, F. O., Bultynck, G., and Parys, J. B. (2021). A Comprehensive Overview of the Complex World of the Endo- and Sarcoplasmic Reticulum Ca2+-Leak Channels. *Biochimica Biophysica Acta (BBA) - Mol. Cell Res.* 2016, 119020. doi:10.1016/j.bbamrc.2021.119020

Poyyomet, N., Nerbonne, J. M., and Kohl, P. (2016). Cardiac Mechano-Gated Ion Channels and Arrhythmias. *Circ. Res.* 118, 311–329. doi:10.1161/circresaha.115.012062

Mijares, A., Altimirano, F., Kolster, J., Adams, J. A., and López, J. R. (2014). Age-Leybaert, L., Lampe, P. D., Dhein, S., Kwak, B. R., Ferdinandy, P., Beyer, E. C., et al. (2010). Connexins in Cardiovascular and Neurovascular Health and Disease: Pharmacological Implications. *Pharmacol. Rev.* 69, 396–478. doi:10.1124/pr.1115.012062

Stout, C. E., Costantin, J. L., Naus, C. C. G., and Charles, A. C. (2002). Intracellular Calcium Signaling in Astrocytes via ATP Release through Connexin Hemichannels. *J. Biol. Chem.* 277, 10482–10488. doi:10.1074/jbc.m109902200

Ravens, U., and Cerbai, E. (2008). Role of Potassium Currents in Cardiac Arrhythmias. *Europace* 10, 1133–1137. doi:10.1039/euroeu19

Rouhana, S., Farah, C., Roy, J., Finan, A., Rodrigues De Araujo, G., Bideaux, P., et al. (2019). Early Calcium Handling Imbalance in Pressure Overload-Induced Heart Failure with Nearly Normal Left Ventricular Ejection Fraction. *J. Physiol.* 114, 823–832. doi:10.1113/jphysiol.2019.12576-016-0519-3

Zima, A. V., Bovo, E., Bers, D. M., and Blatter, L. A. (2010). Ca2+spark-dependent and -independent Sarcoplasmic Reticulum Ca2+leak in Normal and Failing Rabbit Ventricular Myocytes. *J. Physiol.* 588, 4743–4757. doi:10.1113/jphysiol.2010.197913

Tian, J., and Xie, Z.-j. (2008). The Na-K-ATPase and Calcium-Signaling Microdomains. *Physiology* 23, 205–211. doi:10.1152/physiol.00008.2008

Williams, I. A., and Allen, D. G. (2007). Intracellular Calcium Handling in Ventricular Myocytes from Mdx Mice. *Am. J. Physiology-Heart Circulatory Physiol.* 292, H114–H122. doi:10.1152/ajpheart.00688.2006

Yamaguchi, Y., Iribe, G., Nishida, M., and Naruse, K. (2017). Role of TRPC3 and TRPC6 Channels in the Myocardial Response to Stretch: Linking Physiology and Pathophysiology. *Prog. Biophysics Mol. Biol.* 130, 264–272. doi:10.1016/j.pbiomolbio.2017.06.010

Weisbrod, D. (2020). Small and Intermediate Calcium Activated Potassium Channels in the Heart: Role and Strategies in the Treatment of Cardiovascular Diseases. *Front. Physiol.* 11, 590534. doi:10.3389/fphys.2020.590534

Streiff and Sachse. Sarcolemmal vs. SR Ca2+ Currents. Copyright © 2022 Streiff and Sachse. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Streiff and Sachse. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.