Univariate vs Multivariate Sensitivity Analysis of Calcium Dynamics in Simulated Human Heart Failure

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Abstract

Calcium (Ca^{2+}) homeostasis is essential to assure heart contraction. Heart failure (HF) changes lead to abnormal cardiac function and restoring the altered Ca^{2+} dynamics becomes important to maintain cardiac pump function. The electrophysiological exploration of ionic transport mechanisms facilitates the understanding of how they could modulate Ca^{2+} handling. Multiple studies focusing action potential biomarkers have provided a better understanding of cardiac electrophysiology but these studies do not usually consider changes in Ca^{2+} dynamics. Sensitivity analyses are known as potential methods to systematically study the electrical activity in myocytes and our goal is to compare the results obtained by two slightly different sensitivity analyses performed on a human endocardial action potential model under normal and failing conditions. Univariate and multivariate studies are commonly used in the literature to determine the main modulators of electrophysiological characteristics, but results obtained by both methodologies have not been compared in detail. The measurement and analysis of electrophysiological Ca^{2+} indicators reveals, using both methodologies, the importance of SERCA, followed by NCX and I_{CaL} in modulating these characteristics, and highlights some changes in HF.

1. Introduction

The increasing number of cardiac pathologies is leading to the search of new therapies. The use of pharmacological agents has been historically very important because of their capacity to modify the electrical activity of the heart, preventing or reducing the progress of a pathology. In HF, myocytes undergo an electrophysiological (EP) remodeling [1], among other changes, which alters ionic concentrations and action potentials (APs) [2,3]. The process of excitation-contraction coupling is thus affected, leading to cardiac contraction problems and malignant arrhythmias. The regulation of cardiac contractility by cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) requires a Ca^{2+} flux balance control in HF [4]. Intracellular Ca^{2+} transients (CaTs) experimentally measured in failing myocytes show a depressed amplitude, due to the decreased and increased systolic and diastolic $[Ca^{2+}]_i$ respectively, and a prolonged duration (CaTD) [3,5].

The direct relationship between Ca^{2+} cycling and the most relevant sarcolemmal and/or sarcoplasmic reticulum currents has been experimentally established [6,7], but additional channels and transport mechanisms are involved in determining Ca^{2+} dynamics. EP studies, as well as computer simulations, throw light on how all these mechanisms work. Sensitivity analysis is a powerful tool that allows a rapid assessment of parameter variation, either independently (one at a time) or at the same time (multivariate analysis) and helps in the identification of the most important EP modulators [8,9]. Drug effects can also be evaluated using sensitivity analysis, by simulating variations in different ionic parameters regulating the dynamics of ion channels.

The present work was designed to analyze drug-induced modulation of Ca^{2+} dynamics under different conditions (normal and HF) to complement the experimental results or suggest new experiments that clarify the discrepancies. Two methodologies of *in silico* sensitivity analyses were performed to know how parameter variations could regulate Ca^{2+} handling. Results of both analysis show similarities and differences in determining Ca^{2+} modulators. Differences between both conditions reveal the effect of HF remodeling on drug effectiveness and highlight the most suitable parameters to restore Ca^{2+} levels and contractility in each situation.

2. Methods

The O'Hara et al. ventricular AP model (ORd) [10] was used as the baseline model to simulate the EP activity of an endocardial cell at a basic cycle length of 1000 ms. Small modifications to improve the Na^+ current formulation led to a new version of the model (ORdmm). These changes included an enhancement of the fast current (I_{Na}) changing the steady-state dynamics of the gates m_{ss}, h_{ss}, and j_{ss} [11], and an increase of the late component (I_{NaL}) to match experimental observations [12]. To simulate HF, we introduced the EP remodeling reported in the study of Gomez et al. [1] on ORdmm. Sensitivity analyses were performed by variating the following ionic parameters: I_{Na}, I_{NaL}, the transient outward K^+ current (I_{to}), the L-type Ca^{2+} current (I_{CaL}), the rapid delayed rectifier K^+ current (I_{Kr}), the slow delayed rectifier K^+ current (I_{Ks}), the inward rectifier K^+ current (I_{K1}), the Na^+/Ca^{2+} exchange current (I_{NCX}), the Na^+/K^+ pump current (I_{NaK}), the Ca^{2+} uptake via SERCA pump (J_{SERCA}), the SR Ca^{2+} release flux via RyR (J_{rel}), the SR Ca^{2+} leak (J_{leak}) and the Na^+ background current (I_{Nab}). Parameter variations attempted to reproduce potential drug effects and for simplicity, they only included changes in ionic conductances. The studied EP characteristics, measured from steady-state intracellular Ca^{2+} transients (CaTs), were the Ca^{2+} peak achieved in

systole and the CaT duration at 80% recovery ($CaTD_{80}$), which are altered in HF.

A first simple sensitivity analysis consisted of applying a one-at-a-time variation of -60% and +60% to the baseline value of the selected parameters in the two basic models (ORdmm and ORdmm with HF). This variation is within the variability range considered in other sensitivity studies [8,11]. In this univariate study, sensitivities ($S_{c,p}$) were calculated as the percentage of change of the baseline level of Ca^{2+} indicators when existing a ±60% individual parameter variability [8].

$$D_{c,p,x} = \frac{c_{p,x} - c_{basic}}{c_{basic}} \cdot 100 \quad (1)$$

$$S_{c,p} = \frac{D_{c,p,2} - D_{c,p,1}}{\Delta a} \quad (2)$$

$D_{c,p,x}$ is the percentage of change with "c" being the magnitude of the characteristic "c" when parameter "p" undergoes a change (x=1: -60% and x=2:+60% HF),with respect to the basic model (normal or HF ORdmm), and c_{basic} the value of the same property in the basic model; Δa is the total interval of change of parameter p (equal to 1.2 in this case).

Multivariable regression was the second methodology employed for sensitivity calculation. It is based on a linear regression (eq. (3)) involving all the parameters (X) and attempting to predict each EP indicator (Y). Therefore, sensitivities are represented by regression coefficients (B_{PLS}). Two populations of models (with and without HF), with the selected parameters of the model varying randomly between -60% and +60% its original value, were generated. Parameter values within this range were generated from a log-normal distribution of standard deviation equal to 0.3 (2σ corresponds to 95% of conductances varying between 40% and 160%). Regression coefficients were obtained by applying PLS (partial least squares) as shown in eq. (4), after several transformations, such as log-transformations and normalizations [9,13].

$$Y_{predicted} = X \; x \; B_{PLS} \quad (3)$$

$$B_{PLS} = (X^T X)^{-1} \; x \; X^T x \; Y \quad (4)$$

Relative sensitivities were calculated to compare both sensitivity analyses and parameters highlighted as the most important contributors to each EP characteristic. Relative sensitivities were calculated as the ratio between each sensitivity and the maximum absolute value obtained for a particular indicator.

3. Results and Discussion

In Figure 1, relative sensitivities are represented to compare the univariate and multivariate results, revealing the impact of ORdmm parameters on Ca^{2+} biomarkers. In general, the ±60% modulation of the most important ion currents and transport mechanisms contribute similarly to EP characteristics under normal conditions and HF. However, slight differences highlight the existence of synergy between variables, i.e., the variability of the selected parameters contributes to the effect of a particular transport mechanism on Ca^{2+} indicators. For

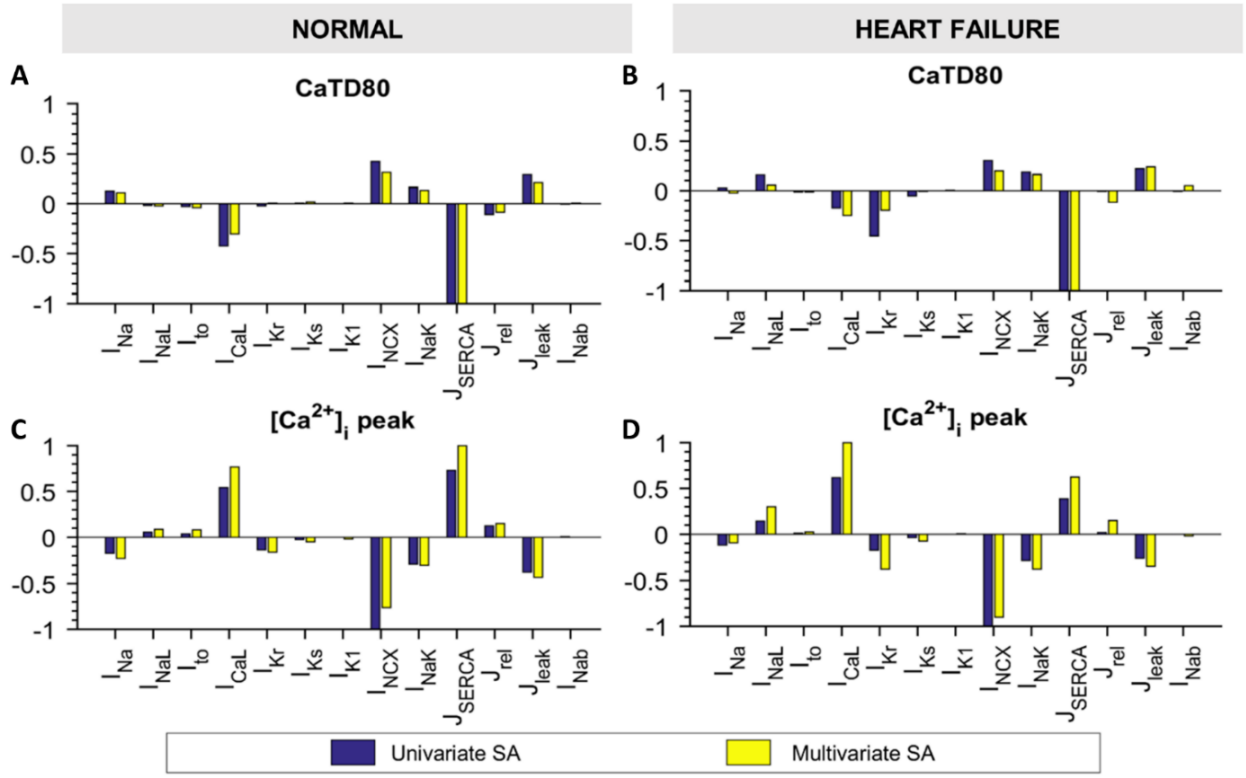

Figure 1. *Univariate vs Multivariate relative sensitivities of CaTD80 and systolic $[Ca^{2+}]_i$ peak to changes in electrophysiological parameters under normal (left panels) and heart failure conditions (right panels).*

example, in Figure 1A, I_{NCX}, I_{CaL} and J_{leak} show a smaller contribution to $CaTD_{80}$ in the multivariable regression than in the univariate analysis under normal conditions. In HF (Figure 1B), the impact of NCX is also smaller in multivariate vs univariate analysis, as well as I_{NaL} effect, but sensitivity to I_{CaL} becomes higher. Multivariate analysis takes into account ionic interactions for a wide HF remodeling variability or when multi-channel drugs are applied, whereas univariate analysis considers the sensitivity of one parameter for a specific and fixed state of the other ion channels. The major difference is observed in I_{Kr} contribution.

The analysis of Ca^{2+} peak modulators reveals differences that lead to a change of the main parameter regulating this biomarker. In Figure 1C, I_{NCX} is the strongest modulator, followed by J_{SERCA} and I_{CaL}, when sensitivities are obtained with the one-at-a-time methodology, whereas multivariate methodology indicates another order of priority: J_{SERCA}, I_{CaL}, and I_{NCX}. In HF (Figure 1D), the strongest modulators are I_{NCX}, I_{CaL} and J_{SERCA}, according to univariate results, whereas the multivariate analysis yields I_{CaL}, followed by I_{NCX} and J_{SERCA} as main modulators.

The main interest of these sensitivity analyses resides in the fact that they reveal how drug-induced changes in ionic channels and transporters modify Ca^{2+} biomarkers. Different effects under normal conditions and HF give an orientation to design experiments with pharmacological agents.

The univariate analysis reveals a high sensitivity of Ca^{2+} biomarkers to J_{SERCA}, being the strongest modulator of $CaTD_{80}$ and the second of Ca^{2+} peak. Absolute sensitivities can be found in Table 1. In HF, these sensitivities are significantly reduced. SERCA ensures intracellular Ca^{2+} uptake inside the sarcoplasmic reticulum (SR), determining SR $[Ca^{2+}]$. Changes in SERCA activity, such as the downregulation experienced in HF, reduces SR Ca^{2+} content. Consequently, Ca^{2+} homeostasis is altered as the measured EP characteristics reflect [3,5]. Pharmacological development to increase SR Ca^{2+} uptake is thus important and can be compared to gene therapy methods [6,14], but targeting J_{SERCA} in HF is hindered by its remodeled activity.

I_{NCX} and I_{CaL} are secondary targets to reduce CaTD, blocking and enhancing them, respectively, although sensitivities to these currents are also reduced in HF. An unexpected finding is the indirect I_{Kr} contribution to $CaTD_{80}$ shortening, only observed in HF (Figure 1A and 1B). This means that an enhancement of I_{Kr}, in addition to decrease APD (parameter very sensitive to this current in the ORd model [10]), could help to decrease CaTD in HF.

Regarding Ca^{2+} peak, as stated above, I_{NCX} is the strongest parameter, followed by J_{SERCA} and I_{CaL}. I_{NCX} activity involves the extrusion of Ca^{2+} out of the cell, reducing intracellular levels. I_{NCX} presents a greater influence than J_{SERCA} in both conditions, becoming an important target in HF to improve CaT properties, although the smaller sensitivity existing under pathological conditions should be taken into account. There are no specific NCX inhibitors, but NCX overexpression showed beneficial results on Ca^{2+} homeostasis in other studies [15]. The relative positive role of I_{CaL} on systolic Ca^{2+} increases in HF because it is not a remodeled ionic mechanism, unlike SERCA or NCX (Figure 1D, blue bars). However, the

	UNIVARIATE				**MULTIVARIATE**			
	CaTD80		**$[Ca^{2+}]_i$ peak**		**CaTD80**		**$[Ca^{2+}]_i$ peak**	
Parameter	**N**	**HF**	**N**	**HF**	**N**	**HF**	**N**	**HF**
I_{Na}	12.03	0.12	-29.61	-7.82	0.10	-0.02	-0.15	-0.05
I_{NaL}	-1.76	4.21	10.38	10.47	-0.02	0.04	0.06	0.17
I_{to}	-2.70	-0.14	7.17	1.10	-0.04	-0.01	0.05	0.02
I_{CaL}	-41.20	-2.84	95.03	43.36	-0.28	-0.19	0.51	0.55
I_{Kr}	-2.05	-11.22	-23.72	-13.13	0.00	-0.14	-0.11	-0.21
I_{Ks}	0.65	-1.48	-4.20	-2.38	0.02	0.00	-0.03	-0.04
I_{K1}	-0.17	0.19	0.20	0.32	0.00	0.00	-0.01	0.00
I_{NCX}	41.17	6.22	-175.09	-68.74	0.28	0.15	-0.50	-0.50
I_{NaK}	16.39	4.47	-51.05	-19.48	0.12	0.13	-0.20	-0.21
J_{SERCA}	-97.59	-24.32	128.24	22.55	-0.91	-0.75	0.66	0.35
J_{rel}	-10.70	-0.09	22.22	1.11	-0.08	-0.09	0.10	0.08
J_{leak}	28.58	5.38	-65.65	-18.27	0.19	0.18	-0.29	-0.19
I_{Nab}	-0.66	-0.17	1.67	0.39	0.00	0.04	0.00	-0.01

Table 1. *Univariate absolute sensitivities (%) and Multivariate regression coefficients of CaTD80 and systolic $[Ca^{2+}]_i$ peak to changes in electrophysiological parameters under normal and heart failure conditions.*

absolute sensitivity is lower in HF because the specific EP remodeling in other parameters affects the capacity of I_{CaL} to increase $[Ca^{2+}]_i$.

Likewise, some regression coefficients of the multivariate analysis decrease in HF in comparison to the impact of parameters under normal conditions. The main parameters regulating $CaTD_{80}$ do not variate with myocyte conditions, except I_{Kr} in the HF model (Figure 1B). Indeed, the multivariate analysis reveals that, although there is a relative increase in I_{Kr} effect, it is small in comparison to univariate results. Specific fixed parameters in HF contribute to the high effect found initially, while considering possible natural or drug-induced variability in the rest of parameters we see less influence. In this case, I_{Kr} could be modulated without great impact on $CaTD_{80}$ in HF, compared to univariate results, whereas in normal conditions there is no effect.

J_{SERCA}, I_{CaL} and I_{NCX} are the main modulators of systolic $[Ca^{2+}]_i$. SERCA has the strongest impact under normal conditions, whereas I_{CaL} becomes the most important modulator in HF. Since L-type Ca^{2+} channels are not affected by EP remodeling, this current maintains its contribution to $[Ca^{2+}]_i$, and the results also suggest an independency of I_{CaL} to the other parameters of the model. This means that an enhancement of I_{CaL} could be beneficial to increase Ca^{2+} peak in HF, regardless of the state of the other parameters. Unexpectedly, I_{NCX} impact does not change between both conditions despite the enhancement experienced in HF.

Due to the minor differences between the single and multivariable regression, the first analysis, which is simpler, is powerful enough to have a general idea of the contribution of ionic parameters.

4. Conclusions

Univariate and Multivariate results agree that an enhanced SERCA activity is beneficial for both the CaTD and the systolic $[Ca^{2+}]_i$. But a further improvement of Ca^{2+} peak is achieved with NCX inhibition or I_{CaL} enhancement because in HF, they are better regulators than SERCA.

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