Optimization of Voltage Protocol to Identify Ion Channel Kinetics

Young-Seon Lee[1, 2], Minjun Lee[1], Jaekyung Song[2], Chae Hun Leem[2, 3*]

[1]Department of Applied Mathematics and Statistics, The State University of New York, South Korea, [2]College of Medicine, University of Ulsan, South Korea, [3]Department of Physiology, Asan Medical Center, Seoul, Korea, South Korea

Submitted to Journal:
Frontiers in Physiology

Specialty Section:
Computational Physiology and Medicine

Article type:
Original Research Article

Manuscript ID:
1119634

Received on:
09 Dec 2022

Revised on:
18 Dec 2022

Journal website link:
www.frontiersin.org

Conflict of interest statement

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

Author contribution statement

Design and conceptualization of the study: YL and CL; Data analysis: YL, JS, and ML; Software development: ML and YL; Writing and editing: YL and CL; All authors contributed to the revision of the manuscript, read, and approved the submitted version.

Keywords

optimization, ion channel, Voltage protocol, parameter estimation, Parameter sensitivity

Abstract

Word count: 209

Ion channel assay using the patch clamp technique is a delicate and time-consuming procedure. Applying an appropriate voltage protocol should reduce the time and effort required to perform the assay. We aim to develop a method to optimize the voltage protocol using parameter sensitivity analysis of model parameters. Recently, an 8 s sinusoidal voltage protocol was developed by the Mirams group to fit a mathematical model for the rapid delayed rectifier potassium current (IKr). This model has eight parameters for ion channel kinetics and one for conductance. A parameter sensitivity analysis was performed by randomly varying eight parameters at evenly spaced time points in the voltage protocol. The relative cumulative sensitivity of the parameters was calculated at various time points, which showed no increase in sensitivities between 2 and 3 s and between 7 and 8 s of the entire protocol. This result suggests that the eight parameters contributed little to the change in the IKr current during the aforementioned two analysis periods. This result prompted us to test whether these two time intervals were necessary for parameter estimation. We compared the fitted parameters and found no difference between the 8 s and 6 s protocols. Therefore, we suggest that parameter sensitivity analysis be applied to optimize voltage protocol.

Contribution to the field

One of the major problems with mathematical models is how to calibrate the model parameters. The traditional approach using square-wave voltage-clamp protocols to obtain experimental data for ion currents is a time-consuming procedure. More recently, Mirams's group developed a shorter voltage protocol that fits mathematical models of ionic channels, and this approach outperformed existing voltage protocols. In this study, we propose an approach to further reduce the duration of the voltage protocol by applying the parameter sensitivity analysis results of the model parameters.

Funding statement

This research was supported by a grant (22213MFDS392) from the Ministry of Food and Drug Safety in 2022. This work was supported by the National Research Foundation of Korea (NRF) research grant funded by the Korean government (MSIT) (2021R1F1A1063218 to YSL).

Ethics statements

Studies involving animal subjects
Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects
Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data
Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: Publicly available datasets were analyzed in this study. This data can be found here: Beattie, K.A., Hill, A.P., Bardenet, R., Cui, Y., Vandenberg, J.I., Gavaghan, D.J., et al. (2018). Sinusoidal voltage protocols for rapid characterisation of ion channel kinetics. J Physiol 596(10), 1813-1828. doi: 10.1113/JP275733..

Optimization of Voltage Protocol to Identify Ion Channel Kinetics

Young-Seon Lee[1,2], Minjun Lee[2], Jaekyung Song[1,3], Chae-Hun Leem[1,3]

[1]Department of Physiology, University of Ulsan, College of Medicine, Seoul, Korea

[2]Department of Applied Mathematics and Statistics, The State University of New York, Korea, Incheon 21985, Korea

[3]Department of Physiology, Asan Medical Center, 88, Olympic-ro 43-gil, Songpa-gu, Seoul, Republic of Korea

*** Correspondence:**
Chae Hun Leem
leemch@gmail.com

Keywords: Optimization, Ion Channel, Voltage Protocol, Parameter estimation, Parameter Sensitivity

Number of words: 1181

Number of figures: 4

Number of tables: 0

Abstract

Ion channel assay using the patch clamp technique is a delicate and time-consuming procedure. Applying an appropriate voltage protocol should reduce the time and effort required to perform the assay. We aim to develop a method to optimize the voltage protocol using parameter sensitivity analysis of model parameters. Recently, an 8 s sinusoidal voltage protocol was developed by the Mirams group to fit a mathematical model for the rapid delayed rectifier potassium current (I_{Kr}). This model has eight parameters for ion channel kinetics and one for conductance. A parameter sensitivity analysis was performed by randomly varying eight parameters at evenly spaced time points in the voltage protocol. The relative cumulative sensitivity of the parameters was calculated at various time points, which showed no increase in sensitivities between 2 and 3 s and between 7 and 8 s of the entire protocol. This result suggests that the eight parameters contributed little to the change in the I_{Kr} current during the aforementioned two analysis periods. This result prompted us to test whether these two time intervals were necessary for parameter estimation. We compared the fitted parameters and found no difference between the 8 s and 6 s protocols. Therefore, we suggest that parameter sensitivity analysis be applied to optimize voltage protocol.

1 Introduction

Mathematical modeling and simulation of cardiac electrophysiology provide insights and predictions into the cellular mechanisms of the cardiac action potential, a key component controlling a wide range of physiological conditions in cardiac function (Rudy, 2004; O'Hara et al., 2011).

When building mathematical models elucidating the mechanisms underlying the physiological functions of cardiac cells, determining a unique set of parameters in the ionic current model is an essential step that requires calibration based on experimental data (Chis et al., 2016; Moreno et al., 2016; Whittaker et al., 2020). Conventional calibration methods have used voltage-clamp protocols, including steady-state activation, inactivation, and recovery from inactivation (Teed and Silva, 2016; Asfaw and Bondarenko, 2019). Miram's group recently proposed that a sinusoidal voltage-clamp protocol can be used as an alternative to conventional voltage protocols for identifying parameters in most of the rapid delayed rectifier potassium current (I_{Kr}) models (Beattie et al., 2018; Lei et al., 2019; Whittaker et al., 2020). Additionally, on validation, their new voltage-clamp protocol demonstrated durable performance in response to the action potential voltage clamp (Beattie et al., 2018).

Recently, parameter sensitivity analysis (PSA) has become more important in various fields for identifying parameters, gaining biological insights, and reducing redundant parameters (Fink and Noble, 2009; Sarkar et al., 2012; Lee et al., 2013; Sher et al., 2013). PSA can identify a subset of redundant model parameters with low sensitivity to the outputs. In this study, we developed an approach to further reduce the time duration of the voltage protocol by applying the findings of parameter sensitivity analysis of the model parameters.

2 Materials and Methods

In this study, we used the mathematical model of hERG channel kinetics described by Beattie et al. (Beattie et al., 2018). This model has eight model parameters $P_1, P_2, \cdots, P_8$ and one conductance parameter $P_9 = G_{k_r}$, where G_{k_r} is the maximal conductance of the hERG channel current $I_{k_r} = G_{kr}[O](V - E_k)$, $[O]$ represents the open probability, and E_k represents the Nernst potential.

$$\frac{d[C]}{dt} = -(k_1 + k_3)[C] + k_2[0] + k_4[IC] \qquad (1),$$

$$\frac{d[0]}{dt} = -(k_2 + k_3)[0] + k_1[c] + k_4[I] \qquad (2),$$

$$\frac{d[I]}{dt} = -(k_2 + k_x)[I] + k_3[0] + k_1[IC] \qquad (3),$$

where $[IC] = 1 - ([C] + [0] + [I])$.

To measure the effect of model parameters on the current, we simulated the model by randomly perturbing the model parameters a thousand times and performed a PSA (Sarkar et al., 2012; Lee et al., 2013) by randomly varying eight parameters except for the conductance parameter. The randomly varying parameters were entered into an input matrix **X** with dimensions 1000 (trials) by 8 (parameters). I_{Kr} currents were calculated at 32 equally spaced time points, starting at 100 ms and ending at 7850 ms at equal intervals of 250 ms. These values were stored as the output matrix **Y** with a dimension of 1000×32. The correlation between **X** and **Y** was calculated by linear regression, resulting in an 8×32 dimension B matrix such that **X**×**B**≈**Y**.

For each parameter, the relative cumulative parameter sensitivity (CPS) at the time point t_k was defined as follows:

$$CPS(t_k) = \frac{\sum_{i=1}^{k} |B_j(t_i)|}{\sum_{i=1}^{32} |B_j(t_i)|},$$

where $k = 1,2,\cdots,32$ and $j = 1,2,\cdots,8$

We followed the method used by Clerx et al. [14] to fit the model parameters. We generated synthetic I_{Kr} data by adding a random noise with a standard deviation of 20 to the I_{Kr} current based on the initial parameters $P_1 = 2.26e^{-4}, P_2 = 0.0699, P_3 = 3.45e^{-5}, P_4 = 0.05462, P_5 = 0.0873, P_6 = 8.91e^{-3}, P_7 = 5.15e^{-3}, P_8 = 0.03158, P_9 = 0.1524$.

Using the synthetic data, we ran a global optimization procedure using the CMA-ES algorithm to find nine model parameters and repeated the same procedure 50 times. In the next step, we used a reduced 6 s sinusoidal voltage protocol to find the model parameters. The optimized model parameters were obtained and compared to the original model parameters.

3 Results

First, we displayed the sinusoidal voltage clamp protocol (Fig. 2A) (the original 8 s protocol) developed by Beattie et al. (Beattie et al., 2018) and the simulated I_{Kr} current (Fig. 2B) using the four-state kinetic model of I_{Kr}, and we plotted these results alongside the experimental data from Beattie et al. (Beattie et al., 2018). In the simulation, we used the same set of model parameters that Beattie et al. used in their study to fit the model.

Next, we sought to determine the optimal length of the voltage protocol used to fit the nine model parameters in the kinetic model of I_{Kr}. Therefore, we generated 1000 population of models of I_{Kr} channel current by perturbing the eight model parameters with a sinusoidal voltage protocol. Although the conductance parameter P_9 was fitted for the model, it was not used to produce the population model because it is different from other types of parameters. Therefore, we only focused on the sensitivity of I_{Kr} current to the kinetic parameters.

The simulated results were used to analyze the parameter sensitivity at each of the 32 time points, from 100 ms to 7850 ms, with equal intervals of 250 ms. Parameter sensitivity was found at each time point (Fig. 3A). It was interesting to find that P_4 showed the highest sensitivity to changes in current. In addition, it was observed that the model parameters exhibited no sensitivity across the time intervals from 2 to 3 s and from 7 to 8 s. Therefore, to find the global effect of the model parameters on the currents, we calculated and plotted the cumulative sensitivity of each parameter (Fig. 3B). The cumulative sensitivity exhibited minimal variation between 2 and 3 s and between 7 and 8 s. This result suggests that none of the eight parameters contributed across the two aforementioned time intervals. Hence, we examined whether these two time intervals are required for parameter estimation using a sinusoidal voltage protocol for optimizing voltage protocol. In other words, we hypothesized that parameter estimation using a time-reduction protocol might be sufficient to determine model parameters. Therefore, we shortened the voltage protocol by eliminating the two time intervals between 2 and 3 s and 7 and 8 s. To compare the difference in parameter estimation between the original protocol (8 s) and the reduced protocol (6 s), we generated synthetic I_{Kr} data with a small amount of noise by using the originally fitted model parameters used in Fig. 2 and fitted nine model parameters using the original 8 s protocol and 6 s protocol, respectively. The global optimization fitting method (CMA-ES) was run 50 times for each case. The fitted model parameters were compared using scatter plots (Fig. 4). The results showed little difference between the two cases (Fig. 4).

4 Discussion

In this study, we proposed a new method to optimize the duration of the voltage-clamp protocol. Beattie et al. developed a new voltage protocol that uses a sinusoidal wave to fit model parameters, which is a more simplified voltage protocol than the conventional voltage protocol that has been used since Hodgkin–Huxley. However, their paper did not clearly explain whether the proposed protocol is optimal for identifying model parameters.

Our approach using parameter sensitivity analysis showed that the optimized parameter set of the ion channel model using the reduced voltage-clamp protocol was nearly identical to the original model with the full-length voltage-clamp protocol.

A previous study by Fink and Noble (Fink and Noble, 2009) also applied parameter sensitivity analysis to reduce the length of voltage protocols. However, they used a generalized sensitivity (Thomaseth and Cobelli, 1999) that differs from our cumulative parameter sensitivity. Additionally, Sher et al. (Sher et al., 2013) applied singular value decomposition based on local sensitivity analysis to identify insensitive and redundant parameters.

Our method can be applied to reduce the duration of various types of voltage-clamp protocols. This approach provides a procedure for systematically reducing a given voltage-clamp protocol to identify model parameters. It is also possible to extend the approach early in voltage-clamp protocol development.

5 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.

6 Author Contributions

Design and conceptualization of the study: YL and CL; Data analysis: YL, JS, and ML; Software development: ML and YL; Writing and editing: YL and CL; All authors contributed to the revision of the manuscript, read, and approved the submitted version.

7 Funding

This research was supported by a grant (22213MFDS392) from the Ministry of Food and Drug Safety in 2022. This work was supported by the National Research Foundation of Korea (NRF) research grant funded by the Korean government (MSIT) (2021R1F1A1063218 to YSL)

8 Acknowledgments

We thank the Ministry of Food and Drug Safety for the financial support and support of our research.

9 Data Availability Statement

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

10 References

Asfaw, T.N., and Bondarenko, V.E. (2019). A Mathematical Model of the Human Cardiac Na(+) Channel. *J Membr Biol* 252(1)**,** 77-103. doi: 10.1007/s00232-018-00058-x.

Beattie, K.A., Hill, A.P., Bardenet, R., Cui, Y., Vandenberg, J.I., Gavaghan, D.J., et al. (2018). Sinusoidal voltage protocols for rapid characterisation of ion channel kinetics. *J Physiol* 596(10)**,** 1813-1828. doi: 10.1113/JP275733.

Chis, O.T., Villaverde, A.F., Banga, J.R., and Balsa-Canto, E. (2016). On the relationship between sloppiness and identifiability. *Mathematical Biosciences* 282**,** 147-161. doi: 10.1016/j.mbs.2016.10.009.

Fink, M., and Noble, D. (2009). Markov models for ion channels: versatility versus identifiability and speed. *Philos Trans A Math Phys Eng Sci* 367(1896)**,** 2161-2179. doi: 10.1098/rsta.2008.0301.

Lee, Y.S., Liu, O.Z., Hwang, H.S., Knollmann, B.C., and Sobie, E.A. (2013). Parameter sensitivity analysis of stochastic models provides insights into cardiac calcium sparks. *Biophys J* 104(5)**,** 1142-1150. doi: 10.1016/j.bpj.2012.12.055.

Lei, C.L., Clerx, M., Gavaghan, D.J., Polonchuk, L., Mirams, G.R., and Wang, K. (2019). Rapid Characterization of hERG Channel Kinetics I: Using an Automated High-Throughput System. *Biophysical Journal* 117(12)**,** 2438-2454. doi: 10.1016/j.bpj.2019.07.029.

Moreno, J.D., Lewis, T.J., and Clancy, C.E. (2016). Parameterization for In-Silico Modeling of Ion Channel Interactions with Drugs. *PLoS One* 11(3)**,** e0150761. doi: 10.1371/journal.pone.0150761.

O'Hara, T., Virag, L., Varro, A., and Rudy, Y. (2011). Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput Biol* 7(5)**,** e1002061. doi: 10.1371/journal.pcbi.1002061.

Rudy, Y. (2004). From genetics to cellular function using computational biology. *Ann N Y Acad Sci* 1015**,** 261-270. doi: 10.1196/annals.1302.022.

Sarkar, A.X., Christini, D.J., and Sobie, E.A. (2012). Exploiting mathematical models to illuminate electrophysiological variability between individuals. *J Physiol* 590(11)**,** 2555-2567. doi: 10.1113/jphysiol.2011.223313.

Sher, A.A., Wang, K., Wathen, A., Maybank, P.J., Mirams, G.R., Abramson, D., et al. (2013). A local sensitivity analysis method for developing biological models with identifiable parameters: Application to cardiac ionic channel modelling. *Future Generation Computer Systems-the International Journal of Grid Computing and Escience* 29(2)**,** 591-598. doi: 10.1016/j.future.2011.09.006.

Teed, Z.R., and Silva, J.R. (2016). A computationally efficient algorithm for fitting ion channel parameters. *MethodsX* 3**,** 577-588. doi: 10.1016/j.mex.2016.11.001.

Thomaseth, K., and Cobelli, C. (1999). Generalized sensitivity functions in physiological system identification. *Ann Biomed Eng* 27(5)**,** 607-616. doi: 10.1114/1.207.

Whittaker, D.G., Clerx, M., Lei, C.L., Christini, D.J., and Mirams, G.R. (2020). Calibration of ionic and cellular cardiac electrophysiology models. *Wiley Interdiscip Rev Syst Biol Med* 12(4)**,** e1482. doi: 10.1002/wsbm.1482.

11 Figure Legends

Figure 1. Structure of Markov model representation of I_{Kr} model. This model has four states IC, I, O, and C, with transition rates $k_1 = P_1\, exp(P_2 v)$, $k_2 = P_3\, exp(-P_4 v)$, $k_3 = P_5\, exp(P_6 v)$, $k_4 = P_7\, exp(-P_8 v)$.

Figure 2. (A) Sinusoidal voltage-clamp protocol, and (B) simulated (red ink) and recorded (blue ink) I_{Kr} current

Figure 3. (A) Heat map representations of parameter sensitivities of eight parameters at 32 time points; MATLAB color map (HSV) was used for the presentation (B) Relative cumulative parameter sensitivities of I_{Kr} current model with the sinusoidal voltage-clamp protocol.

Figure 4. Comparison of parameter fitting results between the original protocol and reduced protocol.

Figure 1.JPEG

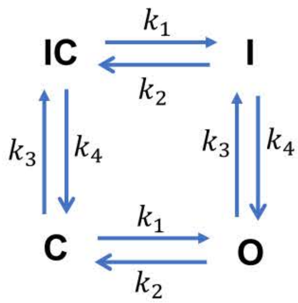
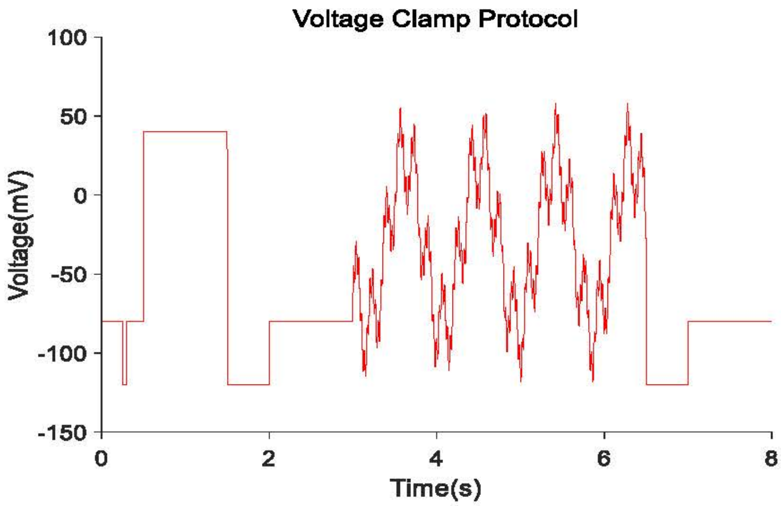


Figure 2.JPEG

A



B

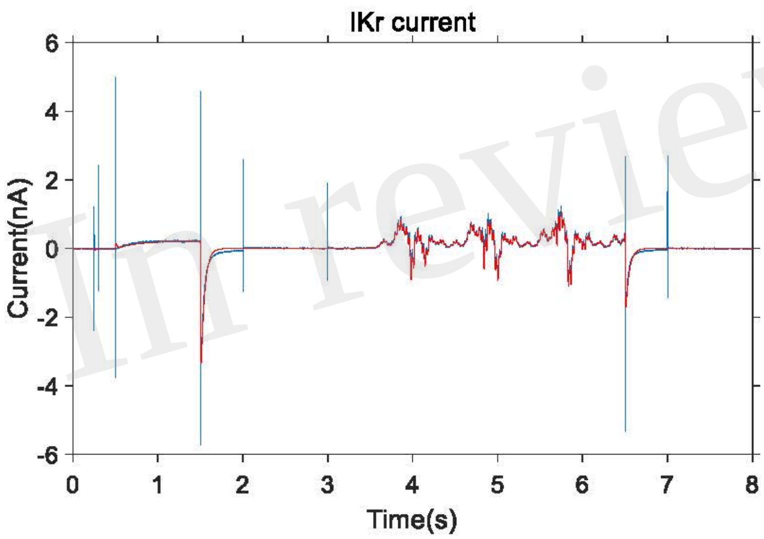
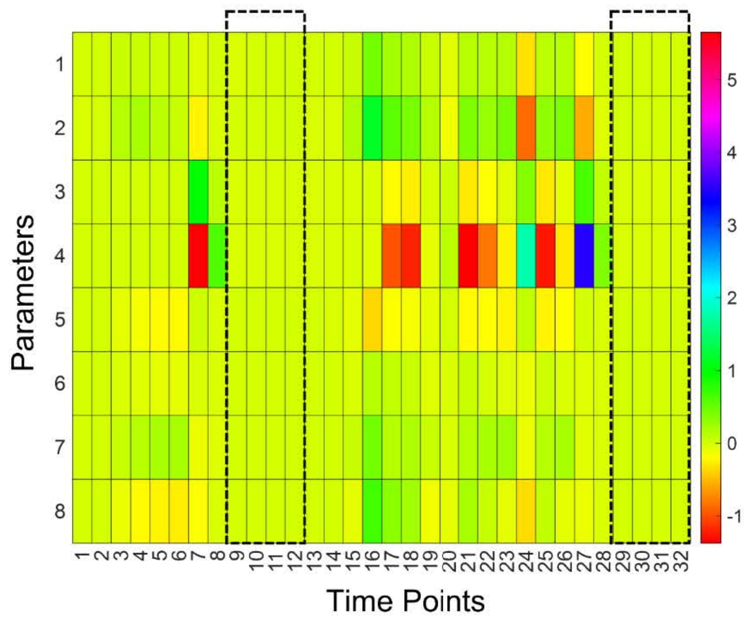


Figure 3.JPEG

A



B

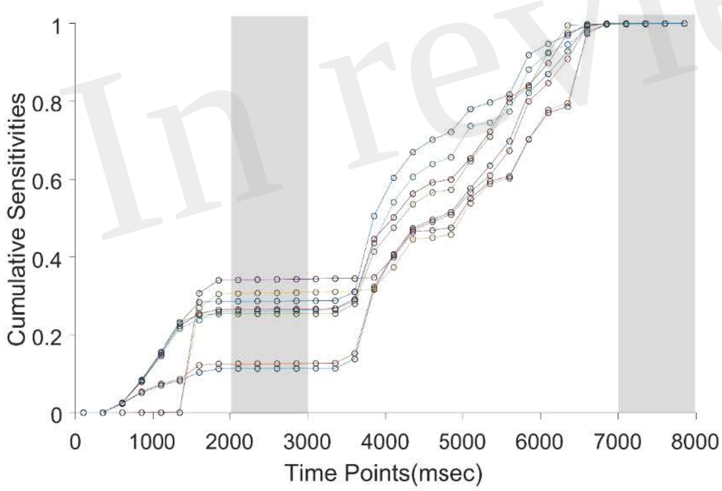


Figure 4.JPEG