Simulations of the Cardiac Action Potential Based on the Hodgkin-Huxley Kinetics with the Use of Microsoft Excel Spreadsheets

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Abstract

The purpose of this study was to develop a method to simulate the cardiac action potential using a Microsoft Excel spreadsheet. The mathematical model contained voltage-gated ionic currents that were modeled using either Beeler-Reuter (B-R) or Luo-Rudy (L-R) phase 1 kinetics. The simulation protocol involves the use of in-cell formulas directly typed into a spreadsheet. The capability of spreadsheet iteration was used in these simulations. It does not require any prior knowledge of computer programming, although the use of the macro language can speed up the calculation. The normal configuration of the cardiac ventricular action potential can be well simulated in the B-R model that is defined by four individual ionic currents, each representing the diffusion of ions through channels in the membrane. The contribution of Na^+ inward current to the rate of depolarization is reproduced in this model. After removal of Na^+ current from the model, a constant current stimulus elicits an oscillatory change in membrane potential. In the L-R phase 1 model where six types of ionic currents were defined, the effect of extracellular K^+ concentration on changes both in the time course of repolarization and in the time-independent K^+ current can be demonstrated, when the solutions are implemented in Excel. Using the simulation protocols described here, the users can readily study and graphically display the underlying properties of ionic currents to see how changes in these properties determine the behavior of the heart cell. The method employed in these simulation protocols may also be extended or modified to other biological simulation programs.

Key Words: simulation, cardiac action potential, Excel, mathematical model

Introduction

Much has been known about the heart cell, particularly about the fundamental mechanisms of voltage and ion regulation. When mathematical expressions for these mechanisms are collected together into differential equations and simulated, the resulting behavior can closely resemble that of the real cell. Half a century ago, Hodgkin and Huxley developed an elegant theoretical model of excitability in nerves (9), the success of which prompted attempts to modify this model for the heart cell (1, 15, 26).

Studies of the electrophysiology of the heart cells have been motivated by both clinical and theoretical considerations (10, 19). However, the complexity of cardiac excitation indeed imposes greater computational demands for simulation of the properties of this tissue, as compared with that for the nerve axon. The fact is that the time course of the cardiac process is slower, there are more components to the currents, and the formulations are more involved than in the axon model. The algorithm of recalculation for the ionic currents depends on the particular model of the heart cells. For example, the Beeler and Reuter (B-R) model for the ventricular heart cell is composed of four individual ion currents considered (1), the Luo-Rudy (L-R) phase 1 model contains six types of ionic currents (15), and other models have postulated

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as many as eight to twelve (6, 14, 16, 27). Generally, these models describe the properties of various ion channels functionally expressed in the cardiac cell membranes.

There are several programs available including Neurosim (21), Neuron (8), Genesis (2), ScoP (13) and Heart 4.X (12), which are ideally suited to the task of modeling the cardiac action potential. However, the methodology presented here offers a cheap alternative. Previous reports have demonstrated that a flexible way of simulating action potential is to use Microsoft Excel to simulate biological systems, such as the action potential (3). An advantage of this method is that this program is included in the computer package as part of Microsoft Office and found in most laboratories, and thus no additional expense is required. This spreadsheet application has been primarily established for business use, but it is often used in science as well. Excel offers a user friendly interface, flexible data manipulation, built-in mathematical functions and instantaneous charting of scientific data. The objective of this study was to describe a method to simulate and investigate the cardiac action potential using Excel.

In the forms of the present simulations as described previously (1, 15), the Hodgkin-Huxley formalism and differential equations were used to reproduce the currents responsible for the cardiac action potential. The simulations allow the user to conduct several "virtual" experiments to determine the effect of different parameters on the model performance. As interest in the field of electrophysiology continues to grow, and as an increasing number of scientists recognize the connection between modeling and experimental studies (19), one may anticipate that this paper will enhance the power of realistic simulations.

Materials and Methods

Full details of the computational methods illustrated in this study have been described previously (1, 15). The objective of the simulation was to determine how the membrane potential of a model heart cell containing various ionic currents responds to a variety of current stimuli. In other words, it is a current clamp simulation in which changes in membrane potential can be modeled in response to constant current stimulus. When the initial membrane potential is specified, the amplitude and duration of current injection chosen, numerical values of model parameters provided, and a series of equation describing the rate constants precisely set, the simulation on change in membrane potential begins. Hodgkin-Huxley-type rate constants are evaluated for the existing membrane potential (9). In current clamp simulations, changes in membrane potential (V) over time are given by:

$$\frac{dV}{dt} = \frac{-I_{total}}{C_m}$$

where C_m is the membrane capacitance and I_{total} is the algebraic sum of ionic currents.

In the simulation based on the B-R and the L-R phase 1 models: $I_{total} = I_{Na} + I_S + I_X + I_K + I_{inject}$, and $I_{total} = I_{Na} + I_S + I_X + I_{K1} + I_{Kp} + I_b + I_{inject}$, respectively, where I_{Na} is a fast Na^+ current; I_S, a slow inward current; I_X, a time-dependent K^+ current; I_K, a time-independent K^+ current (in the B-R model); I_{K1}, a time-independent K^+ current (in the L-R model); I_{Kp}, a plateau K^+ current; I_b, a time-independent background current; and I_{inject}, an injected current.

The activation or inactivation parameters are described by

$$y = \frac{\alpha_y}{\alpha_y + \beta_y}$$

where y is any gating variable, the rate constants for α_y and β_y can be found elsewhere (1, 15)]. In the L-R phase 1 mode, α_{K1} and β_{K1} of I_{K1} depend on extracellular K^+ concentration (15). All ionic currents are computed for 1 cm^2 of membrane, and membrane capacity is set at 1 $\mu F/cm^2$.

The simulation protocol described in this paper, to conduct the mathematical model in a spreadsheet of Microsoft Excel (Redmont, WA, USA), was carried out on Twinhead Pentium-4 computer (Taipei, Taiwan) running under Microsoft Windows XP and Excel 2002. The integration algorithm used to solve the differential equation is simply based on the Euler method. For the sake of clarity, the figures shown in this paper were made in the ORIGIN 6.0 software package (Microcal Software, Inc., Northampton, MA, USA).

Results

Simulated Action Potential Based on the B-R Model

Fig. 1 illustrates the calculated time course of a cardiac action potential and the changes in underlying ionic currents. The simulation protocol run under an Excel spreadsheet was computed from the B-R model. In Fig. 1B, I_{Na} was carried by Na^+ ions. This current reached the peak value of -145 μA at about 1 ms after the current injection. The behavior of this component was very similar to that of the Na^+ current present in the axon because it could be blocked by tetrodotoxin and influenced by extracellular Na^+ concentrations.

The I_S, which has been referred to be L-type Ca^{2+} current (11, 24), rose toward a sustained peak value of -4.5 μA. Its time course was dictated by activation and inactivation factors which were voltage-

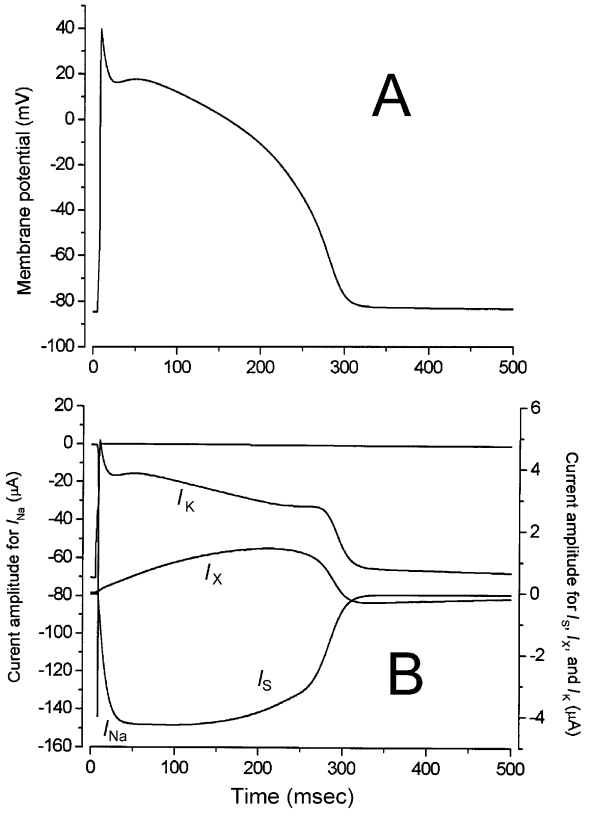

Fig. 1. Computed action potential in response to a 5-ms current pulse of 7-μA amplitude. The simulation was performed according to the B-R model for the ventricular cell. Panel A contains membrane potential on a 0-500 msec scale, whereas panel B shows the corresponding four types of ionic currents. The two inward currents were the fast current Na^+ (I_{Na}) and the slow secondary current labeled I_S. In this and the following figures, inward current was plotted downward. The outward currents, both of which are attributed to K^+, are the time-independent I_K, and the voltage- and time-dependent I_X.

and time-dependent so that the current changes lag behind the voltage changes. A major component of this current was considered to be Ca^{2+}. The I_S was a major inward current in the slow action potential. It is known that adrenergic and cholinergic transmitters, which influence the level of intracellular cyclic AMP inside the heart cell, have a strong effect on this component (18, 24). As the action potential progressed, the falling value of this current was explained by its inactivation factor.

Two outward currents were considered in this model and were carried by K^+ ions, i.e., I_K and I_X. The I_K was a non-linear function of membrane potential that changed instantaneously. Thus in the simulation, its time course would follow changes in membrane potential without time delays. Such outward current tended to moderate the potential changes resulted from the two inward currents. Because of its time-dependent nature, the I_X, also carried by K^+ ions, lagged behind the membrane potential and reached a peak amplitude of 1.5 μA at 220 ms. This current which could be enhanced by β-adrenergic stimulation with isoproterenol (7), contributed to the net outward current responsible for repolarization.

In the B-R model, the sum of the four described currents determined the charge transferred across the membrane and thus the change in membrane potential. When the net current was inward, as with Na^+ activation during the first 3 ms, the membrane was depolarized. When the total current was small, there was no change in potential, as during the plateau. When the total ionic currents flowing outward were larger than those flowing inward, repolarization occurred. The notch at the peak of the action potential was explained in the B-R model by the balance between the rising inward current and the steady K^+ outward current. The repolarization process depended on the deactivation of I_S so that it became smaller than the sum of the two K^+ currents.

Na^+ Current (I_{Na}) and Rate of Depolarization

During the normal depolarization, the total membrane current was completely dominated by the rapid influx of Na^+ ions. Thus, the rate of change in the rising phase of the action potential was often taken as a measure of I_{Na} and its kinetics. The rate of change in membrane potential was an important factor in setting the velocity of propagation in excitable tissue. Thus, it enabled some anti-arrhythmic agents to exert their influences upon conduction by altering the entry of Na^+ ions and the rate of change in membrane potential.

The simulation depicted in Fig. 2 showed how the steady-state potentials determine the amplitude of I_{Na} and the rise of membrane potential in the B-R model. The response started from an initial membrane potential of -84.5 mV, a value at which the activation and inactivation dimensionless variables responded and provided the huge peak in I_{Na} at 1 ms after a current stimulus. This current determined the rate of rise of the potential shown in the lower panel of Fig. 2A. In contrast, the conditions in Fig. 2B where the initial potential was set at -72.5 mV, a value at which the time course of the activation variable has been increased, accounted for the slower rise in the response of I_{Na} which peaked at 1 ms. The reduced I_{Na} resulted in a slower membrane rate of depolarization and a reduced amplitude. As a result, the kinetics of the inactivation of this current can be modified by the initial degree of membrane polarization (10). The properties of the membrane responsiveness immediately following steady-state conditions can be explained by the product of the two dimensionless variables for I_{Na}.

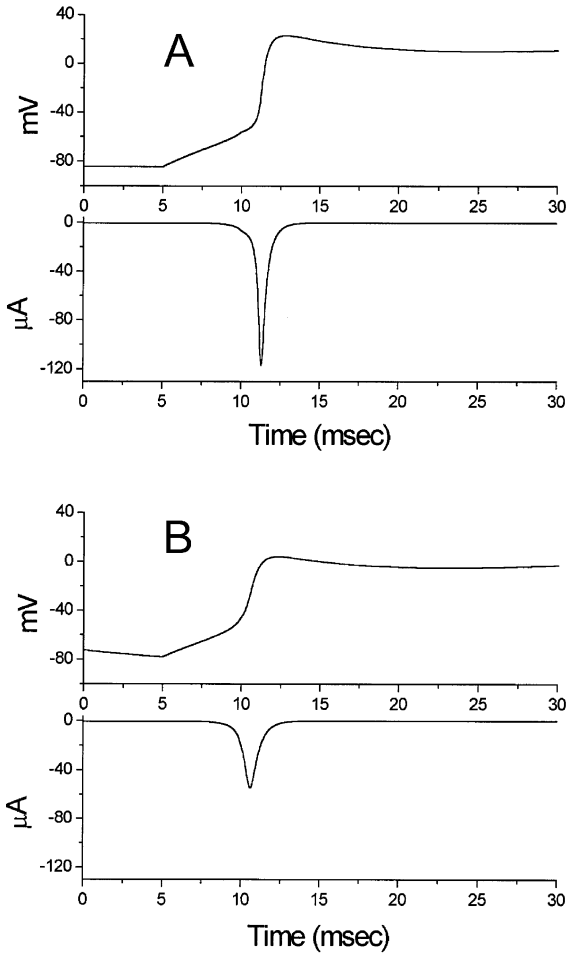

Fig. 2. Computed membrane potential (upper part) and I_{Na} (lower part) in a model cell with the different level of resting membrane potential. Initial membrane potentials shown in panels A and B are -84.5 and -72.5 mV, respectively. The rate of the rise of depolarization in panel A is faster than that in panel B. Accordingly, the amplitude of I_{Na} in panel A is larger than that in panel B. Thus, the level of resting membrane potential will influence the rate of depolarization and the amplitude of I_{Na}. The time scale shown in each panel is 30 ms.

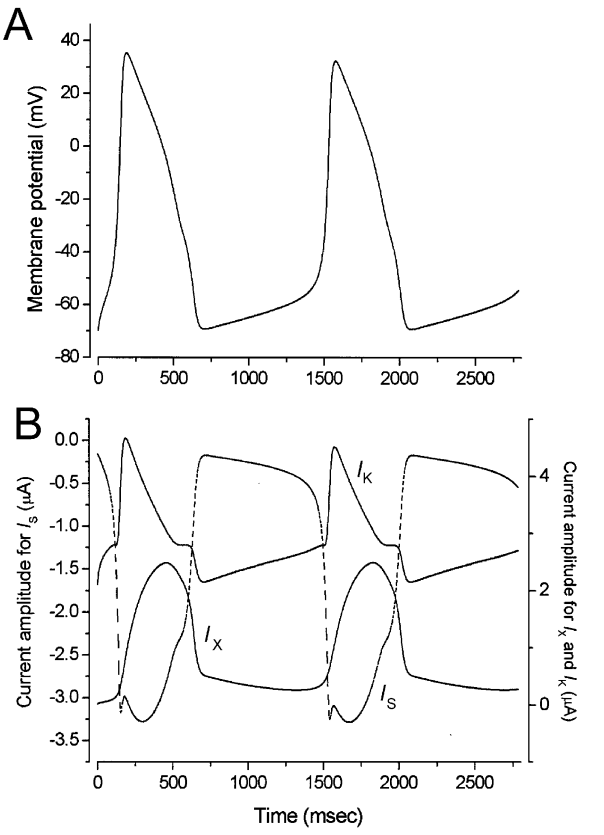

Fig. 3. An oscillatory response produced by a constant depolarizing stimulus of 2.5 μA. Panels A and B are computed membrane potentials and underlying ionic currents, respectively. In this simulation, the component of I_{Na} was excluded. The terms of I_K, I_X, and I_S labeled here are the same as those in Fig. 2.

Slow Action Potential Computed from the B-R Model

Slow action potentials could be generated from membranes in which the Na^+ entry was absent either because of low extracellular concentration or because of the inactivation by membrane depolarization. These responses were calcium action potentials, because they depended on the regenerative entry of I_S. As has been found in certain experimental conditions, the present simulation provided oscillatory behavior for constant depolarization current without the intervention of Na^+ current, which was reminiscent of simulated pacemaker activity in the sinoatrial node cells isolated from the different species (5, 10, 26). Fig. 3 shows a 2.8 s simulation in which the I_{Na} was set to zero and the total ionic current contained a sustained current stimulus of 2.5 μA. In this run, the build-up of intracellular positive charge started the entry of ions via I_S. The depolarization started the lagged K^+ outward current and inactivated the slow inward current. A subsequent increase in I_X and decrease in I_N started the repolarization. During the interval between 0.7 and 1.3 s, the constant stimulus gradually depolarized the membrane again until threshold was reached to start the regenerative slow response at around 1.5 s. The time course in the rate of rise of membrane potential was dependent on the kinetics of the I_S activation and deactivation processes. The magnitude of I_S shown in Fig. 1 and 3 was around 4 μA, about 35 times smaller than that of I_{Na} shown in Fig. 1. The I_S activation and inactivation appeared to be much slower than that of I_{Na}.

Cardiac Action Potential Simulated by the L-R Phase 1 Model

Although the B-R model was based on experimental observations that were available at the time from voltage-clamp studies, the more complex schemes have been developed (14, 15, 16). In order

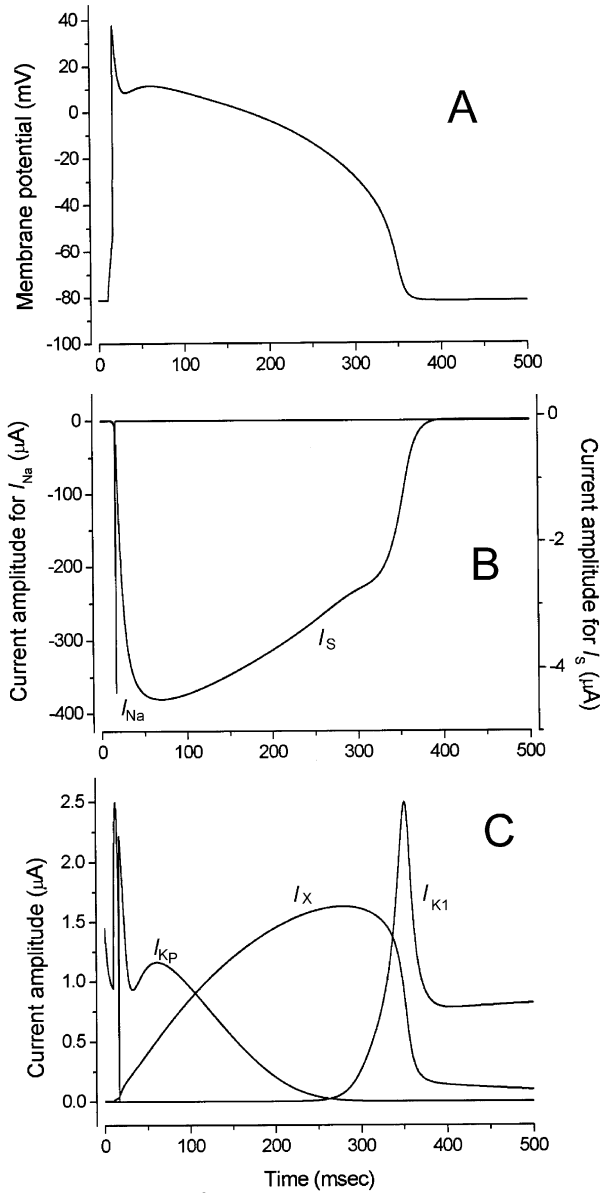

Fig. 4. Computed action potential in response to a 5-ms current pulse of 7-μA amplitude. The simulation was performed according to the L-R phase 1 model for the ventricular cells. Panel A is the change in membrane potential, whereas panels B and C show the corresponding two inward currents (I_{Na} and I_S) and three outward currents (I_X, I_{K1}, I_{Kp}), respectively. Please note, the amplitude of I_{Na} shown here is considerably larger than that in Fig. 2, while the time course and the amplitude of I_X and I_S are similar between these two models.

to verify the simulation protocols run under the spreadsheet of Microsoft Excel, a modified model of the B-R model was further implemented. The formalism was based on the L-R phase 1 model (15). The solutions were also implemented in Excel. Fig. 4A illustrates an action potential computed from this model. The time-independent K^+ current referred to as I_K in the B-R model was modified to exhibit a negativeslope characteristic. This current in the L-R phase 1 model was termed I_{K1}. A novel K^+ current that can be activated at plateau potentials was also incorporated (i.e., I_{Kp}). Another background K^+ current (I_b) was also included (not shown). The time course in individual ionic current during the action potential was illustrated in Fig. 4B. The time course in I_{Na} and I_S computed from the L-R model was similar to that in Fig. 1. However, the amplitude of I_{Na} shown in the L-R model was apparently greater that of the B-R model, although no difference in the peak amplitude of I_S between these two models could be found. I_{Na} present in the L-R model reached a peak amplitude of -370 μA at 1 ms. An abrupt hump occurring during the repolarization process was clearly found in the time course of I_{K1} (15). This property accounts for the negative slope phenomenon of its instantaneous current-voltage relationship during the repolarization. Another current (i.e., I_{Kp}) which was time-independent and responsible for plateau phase, was also incorporated in this model. Unlike that of I_{Na}, the time course and the amplitude of I_X shown in these two models were similar (1, 15). The quantitative difference for the amplitude of I_{Na} reflected that the L-R model contained more realistic I_{Na} kinetics than the B-R model. Accordingly, the rate of depolarization in the L-R model was greater than that in the B-R model.

Another interesting feature found in the L-R phase 1 model was that I_{K1} and I_X of this model could modify their reversal potentials and conductances in response to variations in extracellular K^+ concentration (15). This was important because the increased extracellular K^+ could influence impulse propagation and arrhythmogenesis (20, 22). The ability of changes in extracellular K^+ concentration to exert a strong effect on the level of resting membrane potential and the time course of repolarization was well simulated. As shown in Fig. 5, the resting membrane potential became more positive, while the action potential was shortened and the repolarization was increased, as extracellular K^+ was raised. This change mainly results from an increase in the activation of I_{K1}, which generated an outward current that speeded repolarization. As extracellular K^+ was raised, the amplitude of the total time-independent K^+ current was increased (Fig. 5B). The total time-independent K^+ current was defined as $I_{K1}+I_{Kp}+I_b$. The peak amplitude of the total time-independent K^+ current during the repolarization for 7 mM extracellular K^+ was 2.8 μA, as compared with only 1 μA for 3 mM extracellular K^+.

Discussion

The simulation programs presented here for the modeling of the cardiac action potential may provide many advantages over the previous ones. Many of

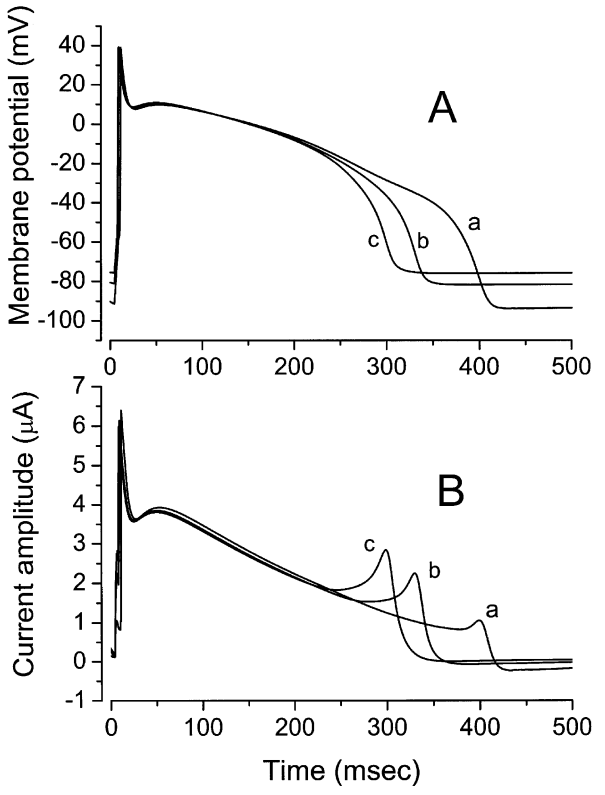

Fig. 5. Effect of extracellular K^+ on the configuration of the cardiac action potentials computed from the L-R phase 1 model. In panel A, as extracellular K^+ increases, action potential duration decreases and resting potential becomes more depolarized. In panel B, the total time-independent K^+ current (i.e., the sum of I_{K1}, I_{Kp} and I_b) was found to depend strongly on extracellular K^+. As the extracellular K^+ concentration rises, the amplitude of the total-time-independent K^+ current that corresponds to the repolarization phase increases. Curves a, b, and c shown in each panel indicate the simulated traces for 3, 5.4, and 7 mM extracellular K^+, respectively.

these advantages result in a dramatic speedup in the process of building and expanding models that usually compensate more than slight reduction in simulation speed. The time taken to build a model is considerably greater than the time taken to actually simulate it, once built. Moreover, adding new components to the simulation does not require rewriting of all the existing codes, as is often the case with dedicated simulations.

One of the purposes in modeling the behavior of the cardiac action potential is to use experimentally obtained data to produce an accurate model that can subsequently be used to forecast unpredicted cell behavior (6,19,27). In the case of the current simulation shown in this study, modeling can be used on Excel spreadsheets to predict the contribution of individual ionic current to the configuration of action potential by modifying that particular current from the simulation, a procedure that may be difficult to be made experimentally. This study demonstrates the feasibility of carrying out solutions on standard equipment readily available to most researchers. Indeed, as interest in the field of cardiac electrophysiology continues to expand, and as increasing numbers of experimentalists recognize the necessary connection between modeling and experimental studies (19), one will anticipate that this paper will promote the power of realistic simulations.

A basic knowledge of both the spreadsheet function and the programming with the built-in macro language is needed in the present protocols. Assuming that the model equations are solved in an identical manner, the method should be comparable to a simulation carried out on specialized softwares (2, 8, 13, 21). Programs are known to use looping structures where data are calculated in a cyclic manner before the calculation is moved to the next stage (3). To compute the simulation protocols shown here, the recalculation function needs to be used to force the spreadsheet to be recalculated row by row. Excel uses an identical procedure with the exception that the product of the calculations is displayed in the spreadsheet and not hidden within the workings of the program. Built-in error messages are also provided in it. As a result, this makes debugging easier in Excel, because the results of the calculations are visible and any nonsensical simulation results can be readily identified. It can thus be expected to be quickly adopted by many users for such simulations shown here.

The use of Excel is advantageous in that we can utilize a variety of powerful tools of Excel for providing databases, figures, documents, graphics, statistical analysis, etc., in the same spreadsheet (3). However, the time to perform the simulations depends not only on the CPU speed of the computer, but also the number of data involved in the calculation and the number of cells containing macro functions. It is also recommended that the recalculation mode in Excel be changed from the 'Automatic' to 'Manual', because the real-time recalculation may be relatively slow.

The method developed in this study provides a feasible alternative, and should enable users to examine the existing models and develop newer ones, without having to acquire knowledge of programming languages. The present approach also allows the user not only to 'visualize' the mechanics of simulation but also to 'see' actually what goes on when model simulation is conducted. A noteworthy difference between the present method and the commercially available simulation software is that neither translation of the model equations into a programming language nor their compilation into an executable program is required in this method.

It is worth mentioning that in the two models used here, inadequate information is available concerning the kinetics important in excitation-con-

traction coupling, the Na^+-Ca^{2+} exchange process, and the electrogenic pumping mechanisms (4, 10, 16). Other types of important K^+ currents (e.g., ATP-sensitive K^+ current, acetylcholine-activated K^+ current, and the rapid and slow components of delayed rectifier K^+ current) are also known to be responsible for the generation of the cardiac action potential (6, 23, 25, 27). More complex model is thus needed to develop in terms of the complexity of the action potentials from the heart. In this regard, because VBA for Excel allows the users to develop custom applications of Microsoft Excel, the spreadsheet combined with this programming language may still have the capability of creating a template and doing such a sophisticated solution as long as the forms of the differential equations are created.

Recent reports have shown that Microsoft Excel could have some flaws on statistical analyses (17). However, no significant change in our simulation made on Microsoft Excel spreadsheets can be observed as compared with previous reports (1, 15). It remains to be clarified to what extent the inaccuracy of the statistical calculations found in Excel affects our simulation approach.

Nevertheless, the simulations presented here are user-friendly and may be solved without any prior knowledge of computer programming. Once an Excel worksheet is written for a class of simulation protocols, running the program is easy. Testing hypotheses by simulating new conditions comes naturally within the familiar context of Excel. The simulations described in this paper provide the insights through which the ionic mechanisms of the cardiac action potential can be elucidated by using an easily understood protocol on Excel. Using Excel programs, kinetic properties of molecular and cellular mechanisms of cardiac electrical activity can also be incorporated into cellular mathematical models of the heart. The method described here can also be modified and fit in other biological simulation programs.

Acknowledgments

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Notes

If the readers feel interested in the original spreadsheet in Excel, please contact the corresponding author.

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