Influence of channel subunit composition on L-type Ca\(^{2+}\) current kinetics and cardiac wave stability

Vadam Gudzenko,1* Yohannes Shiferaw,4* Nicoletta Savalli,1 Roshni Vyas,1 James N. Weiss,2,3 and Riccardo Olcese1

1Department of Anesthesiology, Division of Molecular Medicine, 2Department of Medicine, Division of Cardiology, and 3Cardiovascular Research Laboratory, David Geffen School of Medicine at the University of California Los Angeles, Los Angeles; 4Department of Physics, California State University Northridge, Northridge, California

Submitted 22 October 2006; accepted in final form 29 May 2007

Gudzenko V, Shiferaw Y, Savalli N, Vyas R, Weiss JN, Olcese R. Influence of channel subunit composition on L-type Ca\(^{2+}\) current kinetics and cardiac wave stability. Am J Physiol Heart Circ Physiol 293: H1805–H1815, 2007. First published June 1, 2007; doi:10.1152/ajpheart.01160.2006.—Previous studies have demonstrated that the slope of the function relating the action potential duration (APD) and the diastolic interval, known as the APD restitution curve, plays an important role in the initiation and maintenance of ventricular fibrillation. Since the APD restitution slope critically depends on the kinetics of the L-type Ca\(^{2+}\) current, we hypothesized that manipulation of the subunit composition of these channels may represent a powerful strategy to control cardiac arrhythmias. We studied the kinetic properties of the human L-type Ca\(^{2+}\) channel (Cav1.2) coexpressed with the \(\alpha_\text{1C}\)-subunit alone (\(\alpha_\text{1C} + \alpha_\text{2}\delta\)) or in combination with \(\beta_\text{2a}\), \(\beta_\text{2b}\), or \(\beta_\text{3}\) subunits (\(\alpha_\text{1C} + \alpha_\text{2}\delta + \beta\)), using Ca\(^{2+}\) as the charge carrier. We then incorporated the kinetic properties observed experimentally into the L-type Ca\(^{2+}\) current mathematical model of the cardiac action potential to demonstrate that the APD restitution slope can be selectively controlled by altering the subunit composition of the Ca\(^{2+}\) channel. Assuming that \(\beta_\text{2b}\) most closely resembles the native cardiac L-type Ca\(^{2+}\) current, the absence of \(\beta\), as well as the coexpression of \(\beta_\text{2a}\), was found to flatten restitution slope and stabilize spiral waves. These results imply that subunit modification of L-type Ca\(^{2+}\) channels can potentially be used as an antiarrhythmic strategy.

Ca\(^{2+}\) channel; restitution; cardiac action potential; spiral waves; beta subunit

VENTRICULAR FIBRILLATION (VF) occurs when a propagating electrical wave breaks apart into multiple reentrant spiral waves, as it passes through tissue with nonuniform electrical properties (34). Traditionally, wave break causing VF has been attributed chiefly to preexisting structural and electrophysiological heterogeneity, which is exacerbated by remodeling in the setting of heart disease. However, recent theoretical and experimental studies have shown that wave break can arise from dynamical factors as well (4, 6, 34). A key dynamical factor regulating wave break is the action potential (AP) duration (APD) restitution, which refers to the dependence of APD on the preceding diastolic interval (DI). Experimental and theoretical studies have shown that the initiation and maintenance of VF is strongly influenced by the slope of the APD restitution curve (4, 6), such that a steep restitution curve (slope > 1) destabilizes reentry and promotes wave break. Drugs that flatten the APD restitution curve, such as verapamil and bretylium, have been shown to convert VF to ventricular tachycardia (4, 23, 35) and, in computer simulations, to decrease the vulnerable window for initiation of reentry (20).

The APD restitution curve depends on the time course of ion currents that regulate the voltage across the cell membrane. The predominant inward current during the plateau of the APD is the L-type Ca\(^{2+}\) current, which plays an important role in regulating the shape and kinetics of the AP (5, 21). Critical aspects of the L-type Ca\(^{2+}\) current that influence the APD restitution properties are its rate and degree of inactivation during the AP plateau, and also the rate of recovery from inactivation at the resting membrane potential. Accordingly, we hypothesized that manipulation of the subunit composition of the L-type Ca\(^{2+}\) channel may provide a highly selective means to control the APD restitution characteristics in a therapeutically desirable manner. In this work, we combined experimental and computational approaches to identify components of the Ca\(^{2+}\) channel that can be genetically manipulated to reduce restitution slope.

To explore this possibility we took advantage of the structural complexity of the L-type Ca\(^{2+}\) channel in the heart (Ca\(_{\text{a,1.2}}\)), which consists of a pore-forming \(\alpha_{1C}\)-subunit and the regulatory subunits \(\beta\) and \(\alpha_\text{2}\delta\) (16, 31, 32). Studies have shown that \(\beta\)-subunits (thought to be \(\beta_\text{2}\) in the heart (2)), which bind to the intracellular linker between domains I and II of the \(\alpha_1\)-subunit (17, 18), participate in the trafficking of the pore-forming \(\alpha_{1C}\)-subunit and modulate its biophysical and kinetic properties (1, 10, 13, 14). Although the modulation of voltage-dependent inactivation by \(\beta\)-subunits has been extensively investigated, their modulatory effects under conditions relevant to the shape of the APD restitution curve, using Ca\(^{2+}\) as charge carrier, have not been explored. Thus we present novel data using Ca\(^{2+}\) as charge carrier to preserve Ca\(^{2+}\)-dependent inactivation, to investigate the inactivation and recovery kinetics of four different subunits compositions of L-type channels (\(\alpha_{1C} + \alpha_\text{2}\delta, \alpha_{1C} + \alpha_\text{2}\delta + \beta_\text{2a}, \alpha_{1C} + \alpha_\text{2}\delta + \beta_\text{2b}, \alpha_{1C} + \alpha_\text{2}\delta + \beta_\text{3}\)) of the human Ca\(_{\text{a,1.2}}\) channel expressed in Xenopus laevis oocytes. Although \(\beta\)-subunits have been successfully expressed in adult cardiac myocytes by recombinant adenovirus infection (2, 31), we used the oocyte expression system to be able to precisely control specific channel compositions, including the lack of any endogenous \(\beta\)-subunits (\(\alpha_{1C} + \alpha_\text{2}\delta\)).

*V. Gudzenko and Y. Shiferaw contributed equally to this work.

Address for reprint requests and other correspondence: R. Olcese, Division of Molecular Medicine, BH 570 CHS, Dept. of Anesthesiology, David Geffen School of Medicine, Univ. of California Los Angeles, Box 957115, Los Angeles, CA 90095-7115 (e-mail: rolcese@ucla.edu).

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without the confounding influences of native L-type Ca\(^{2+}\) channel subunits present in cardiac myocytes. To estimate the influence of the subunit composition on the APD restitution properties, we developed a phenomenological model of the L-type Ca\(^{2+}\) current, in which all kinetic properties could be fitted directly to the experimental measurements. Incorporating this model into an established mathematical model of the ventricular AP, we explored the relationship between APD restitution and the kinetics of different subunit compositions, assuming that the \(\beta_{2b}\)-subunit mostly closely resembles the native L-type Ca\(^{2+}\) current (2). On the basis of these studies, we find that the shape of the APD restitution curve is sensitive to subunit composition of the L-type Ca\(^{2+}\) channel, as a consequence of the strong \(\beta\)-subunit modulation of the degree of Ca\(^{2+}\) and voltage-dependent inactivation. Using this AP model we also explored the relationship between spiral wave stability and L-type Ca\(^{2+}\) current kinetics, to show that spiral wave breakup can be eliminated by manipulating the subunit composition of the L-type channels.

**MATERIALS AND METHODS**

**Molecular Biology and Oocyte Preparation**

We have used the human L-type Ca\(^{2+}\) channel \(\alpha_{1c}-\gamma\) (26, 27, 37) and the accessory subunits \(\alpha_\delta, \beta_3, \beta_2a, \beta_2b\) in pBluescript, pAGA2, and pAGA3 vectors (courtesy of N. Qin and N. M. Soldatov). The mRNAs were transcribed in vitro (mMESSAGE mMACHINE, AMBION) and injected in Xenopus laevis oocytes. Oocytes were obtained from adult Xenopus laevis. Xenopus laevis were handled in compliance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Protocols were approved by the University of California, Los Angeles Institutional Animal Care and Use Committee. The frogs were anesthetized by 40-min immersion in 0.1\% tricaine, a portion of the ovary was removed, and stage V and VI oocytes were selected, according to the procedures. One day before mRNA injection, the oocyte follicular layer was removed by collagenase treatment. Frogs were euthanized in 1\% tricaine. A 50-nl dose of cRNA containing 0.2 ng/ml of each subunit was injected per oocyte. We produced four different composition of L-type Ca\(^{2+}\) channel: \(\alpha_1c + \alpha_\delta, \alpha_1c + \alpha_\delta + \beta_2a, \alpha_1c + \alpha_\delta + \beta_2b, \) and \(\alpha_1c + \alpha_\delta + \beta_3.\) The oocytes were maintained at 17–19°C in modified Barth’s solution supplemented with 100 \(\mu\)M penicillin, 50 \(\mu\)M gentamicin, and 2.5 mM pyruvate. Ca\(^{2+}\) channel currents were recorded after incubation for 4–8 days.

**Electrophysiology**

The cut-open oocyte voltage-clamp technique (29) was used to record Ca\(^{2+}\) currents from oocytes expressing \(\alpha_{1c}-\gamma\) in combination with the regulatory \(\alpha_\delta, \beta_2a, \beta_2b, \) and \(\beta_3\) Ca\(^{2+}\) channel subunits. The composition of the external solution (recording chamber and guard compartments) was 10 mM Ca\(^{2+}\), 96 mM Na\(^+\), 10 mM HEPES, titrated to pH 7.0 with methanesulfonic acid. The lower chamber in contact with the front of the oocyte permeabilized with 0.1% saponin, contained 110 mM K-glutamate and 10 mM HEPES titrated to pH 7.0 with NaOH. Before recording, all the oocytes were injected with 100 nl of BAPTA-NA4, 50 mM titrated to pH 7.0 with methanesulfonic acid, to prevent activation of endogenous Ca\(^{2+}\)-activated Cl\(^{−}\) channels (12). To remove contaminating nonlinear charge movement related to the oocytes endogenous Na-K-ATPase (22), 0.1 mM ouabain was added to all external solutions. Leakage and linear capacity currents were compensated analogically and subtracted online using p/4 and p/6 subtraction protocol from –90 mV holding potential. Ca\(^{2+}\) current recordings used to estimate the recovery from inactivation were acquired unsubtracted. Current traces were filtered at 1/5 of the sampling frequency.

**Mathematical Modeling**

A phenomenological model of the L-type Ca\(^{2+}\) channel. To evaluate the effects on the AP of different L-type Ca\(^{2+}\) channel subunit modifications, we have developed a simplified mathematical model of the L-type Ca\(^{2+}\) current and incorporated it into an established ionic model (3) (Fox AP model) of the membrane voltage dynamics, modified to include the Ca\(^{2+}\) cycling model of Shiferaw et al. (24), which more realistically represents Ca\(^{2+}\) cycling dynamics at fast heart rates relevant to tachyarrhythmias. The key feature of this model is that it incorporates our experimentally measured time constants of Ca\(^{2+}\) and voltage-dependent inactivation and recovery, as well as the observation that recovery occurs via a biexponential time course. Furthermore, the kinetic parameters of this model can be chosen to modify the properties of the L-type Ca\(^{2+}\) current formulation in the Fox AP model, which is based on cardiac myocyte data assumed to represent the native \(\beta_{2b}\)-subunit (2). Thus our L-type Ca\(^{2+}\) current model can be used to assess how a given change from the native \(\beta_{2b}\) to another type of \(\beta\)-subunit influences the voltage dynamics of a simulated cardiac myocyte.

The L-type Ca\(^{2+}\) current is formulated as

\[
I_{Ca} = N \cdot d \cdot f_{V Ca} \cdot i_{Ca}
\]

where \(N\) is the number of channels in the cell, \(d\) is the voltage-dependent activation gate variable, \(f_{V Ca}\) is a voltage- and Ca\(^{2+}\)-dependent inactivation gate, and \(i_{Ca}\) is the single channel current. To model Ca\(^{2+}\) and voltage-dependent inactivation and recovery from inactivation, we treat \(f_{V Ca}\) as a sum of two independent gate variables

\[
f_{V Ca} = w f_{V Ca}^i + (1 - w) f_{V Ca}^s
\]

where \(w\) is a weight factor adjusted to fit the relative amplitude of the two experimentally measured recovery components. The gate variables \(f_{V Ca}^i\) and \(f_{V Ca}^s\) model inactivation and recovery from inactivation via a fast and slow pathway, respectively. The time dependence of these gates is written in the usual way

\[
df_{V Ca}^i \over dt = f_{V Ca}^i - f_{V Ca}^s / \tau_{V Ca}^i
\]

with \(i = 1,2,\) and where the steady-state inactivation gates are chosen to have the mathematical form

\[
f_{V Ca}^i = f_{V Ca}^i \cdot f_{V} (1 - f_{V}) + f_0
\]

where \(f_{V Ca}^i\) and \(f_{V}\) are the Ca\(^{2+}\) - and voltage-dependent threshold functions implemented in the Fox AP model. These functions are

\[
f_{V} = \frac{1}{1 + \left(\frac{f_\theta}{f_\gamma}\right)^{w}}
\]

where \(f_\theta\) is the threshold for Ca\(^{2+}\)-dependent inactivation. The variable \(f_0\) is a constant that fixes the minimum steady-state value of \(f_{V Ca}^i\) and determines the pedestal L-type Ca\(^{2+}\) current after inactivation. The two time constants for inactivation and recovery are governed by

\[
\tau_{V Ca}^i = \tau_{in} + (\tau_{fast} - \tau_{in}) \cdot f_{V}^2
\]

so that inactivation occurs with a time constant \(\tau_{in}\) whereas recovery occurs via a fast and slow time constant given by \(\tau_{fast}\) and \(\tau_{slow}\), respectively. Since we observed only one time constant of inactivation we have chosen \(\tau_{V Ca}^i = \tau_{V Ca}\) to be the same (\(\tau_{in}\)) above the activation threshold (voltage \(V > -40\) mV). The L-type Ca\(^{2+}\) current formulation in the Fox AP model can be achieved by setting \(w = 1\), and \(f_0 = 0\), with \(\tau_{in} = \tau_{fast} = 40\) ms so that inactivation is
Produced the typical left shift of the space step was 0.015 cm and the time step varied from 0.1 to we have investigated the role of three different diffusion equation voltage in cardiac tissue, and then delivering the S2 stimulus at varying coupling intervals. The APD after the last stimulus was then graphed vs. the DI of the previous beat.

Two-dimensional tissue simulations. We modeled a two-dimensional (6.75 × 6.75 cm²) monodomain tissue using the reaction-diffusion equation

\[
C_m \frac{\partial V}{\partial t} = -I_m + D \left( \frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2} \right)
\]

where \( C_m = 1 \mu F/cm^2 \) is the transmembrane capacitance, \( D = 5 \times 10^{-4} \text{ cm}^2/\text{ms} \) is the effective diffusion coefficient of membrane voltage in cardiac tissue, and \( I_m \) is the total single cell ionic current density. The reaction diffusion equation was integrated with an operator splitting method and adaptive time step method (19). The space step was 0.015 cm and the time step varied from 0.1 to 0.01 ms.

RESULTS

Functional Expression of \( \alpha_{1C,77} \) and Accessory Subunits

Using the cut-open oocyte voltage clamp technique, we investigated the steady-state and time-dependent properties of the Ca²⁺ current elicited by coexpressing the pore-forming \( \alpha \)-subunit of the L-type channel (Ca₉.1.2) with the modulatory subunits \( \alpha_2\delta \), alone or in combination with \( \beta_2a \), \( \beta_2b \), or \( \beta_3 \) subunits. It is well documented that \( \beta \)-subunits [thought to be the \( \beta_2b \) in native cardiac L-type Ca²⁺ channels (2)] modulate the voltage-dependent inactivation of the Ca²⁺ channel and facilitate channel opening. However, the influence of \( \beta \)-subunit composition on the Ca²⁺-induced Ca²⁺ inactivation properties of the L-type Ca²⁺ channel has not been fully explored. Here, we have investigated the role of three different \( \beta \)-subunits on those kinetic properties of the L-type Ca²⁺ current most relevant to APD restitution, namely the inactivation and recovery kinetics.

The functional expression of the modulatory \( \beta \)-subunits was confirmed by the leftist shift in the voltage dependence of activation revealed by the current-voltage relationship (I-V) in all the \( \alpha + \beta \) combinations, as shown in Fig. 1. Coexpression of any of the \( \beta \)-subunits tested produced on average a ~10-mV shift of the I-V curve toward more negative potentials, compared with \( \alpha_{1C} + \alpha_2\delta \) channels. This voltage shift is well known as a signature of the functional expression of \( \beta \)-subunits (1, 13, 15). The saturating expression of \( \beta \)-subunits was confirmed injecting cRNA encoding for \( \beta_2b \), at 50 and 200% of the concentration used in this study (0.2 mg/ml). Also, we found no significant difference in the peak current \( I_{\text{peak}} \) amplitude and inactivation kinetics when the cRNA encoding for \( \beta_2b \), was injected at 50% (0.1 mg/ml) or 200% (0.4 mg/ml) of the concentration used in this study. \( I_{\text{peak}} \) (0.1 mg/ml) = 108.1 ± 4.6 nA; \( I_{\text{peak}} \) (0.4 mg/ml) = 94.1 ± 18.6 nA; \( I_{\text{peak}} \) (0.2 mg/ml) = 102.2 ± 24.7 nA. Similarly, no significant difference was observed in the time constant of the Ca²⁺ current inactivation during 400 ms depolarization (\( \tau_{\text{inact}} \)) for the three concentration of \( \beta_2b \)-subunits. At +10 mV, \( \tau_{\text{inact}} \) (0.1 mg/ml) = 92.0 ± 7.8 ms, \( \tau_{\text{inact}} \) (0.2 mg/ml) = 82.6 ± 3.5 ms, and \( \tau_{\text{inact}} \) (0.4 mg/ml) = 85.6 ± 3.6 (n = 4 for each concentration ratio, same day, same batch of oocytes), demonstrating that under our conditions (0.2 mg/ml) the experiments are performed at the saturating part of the dose-response curve for \( \beta \)-subunit effect. In fact, \( \beta_2b \) is the largest of the \( \beta \)-subunits used in this study (628aa), giving the smallest (most unfavorable) \( \alpha:\beta \) molar ratio when coexpressed with \( \alpha_{1C} \) (see MATERIALS AND METHODS).

Subunit Composition of the L-type Ca²⁺ Channel Modulates the Rate of Ca²⁺- and Voltage-Induced Inactivation

The \( \alpha_{1C} \)-subunit undergoes Ca²⁺-induced inactivation, which dominates over voltage-induced inactivation as a consequence of a conformational change of the protein mediated by calmodulin (for a recent review, see Ref. 11). Most studies investigating the effects of Ca²⁺ channel modulatory subunits on inactivation have examined only voltage-induced inactivation and have not characterized recovery from inactivation in detail. Using Ca²⁺ as a charge carrier to preserve the Ca²⁺-induced inactivation, we studied the effect of different \( \beta \)-subunits, as well as the absence of a \( \beta \)-subunit, on the inactivation properties of \( \alpha_{1C} + \alpha_2\delta \). Depolarizing pulses (400 ms, slightly longer than a typical physiological cardiac action potential) from a −90 mV holding potential (HP) were used to quantify the effects of the subunit composition on the inactivation properties of the L-type Ca²⁺ current.

The decay of the macroscopic conductance was well fit by a monoexponential function of the form \( I(t) = A \cdot \exp(-t/\tau) + B \) where \( t \) is the time, \( A \) is the amplitude of the inactivating component of the current, \( \tau \) is the time constant of the decay, and \( B \) is the steady-state nonactivating current. The time-dependent component reflected mainly Ca²⁺-induced inactivation, since the voltage-dependent inactivating component measured with Ba²⁺ replacing Ca²⁺ as the charge carrier was much slower (data not shown). Over 400 ms, a duration relevant to the cardiac APD, the second voltage-dependent component was well approximated by a time-independent pedestal.
Representative current recordings during depolarization to −20, 0, and 30 mV are shown in Fig. 2 for \( \alpha_{1C} + \alpha_{2}\delta \) expressed alone (A) and with \( \beta_{2a} \) (B), \( \beta_{2b} \) (C), or \( \beta_{3} \) (D), by the cut-open oocyte voltage-clamp technique. Representative traces are shown for a 400-ms step depolarization to the indicated potentials. Current traces were fit to a monoexponential function \( I_{Ca} = A \cdot e^{-t/\tau} + B \) (solid lines shown superimposed) where \( I_{Ca} \) is calcium current, \( A \) is the amplitude of the inactivating component of the current, \( \tau \) is the time from the beginning of the depolarizing step, \( \tau \) is the time constant of inactivation, and \( B \) is the noninactivating (pedestal) component of the current.

Fig. 2. Subunit composition controls the rate of inactivation of the L-type \( Ca^{2+} \) current. \( Ca^{2+} \) current recordings from oocytes expressing \( \alpha_{1C} + \alpha_{2}\delta \) and \( \alpha_{1C} + \alpha_{2}\delta + \beta_{2a} \) (B), \( \beta_{2b} \) (C), and \( \beta_{3} \) (D), by the cut-open oocyte voltage-clamp technique. Representative traces are shown for a 400-ms step depolarization to the indicated potentials. Current traces were fit to a monoexponential function \( I_{Ca} = A \cdot e^{-t/\tau} + B \) (solid lines shown superimposed) where \( I_{Ca} \) is calcium current, \( A \) is the amplitude of the inactivating component of the current, \( \tau \) is the time from the beginning of the depolarizing step, \( \tau \) is the time constant of inactivation, and \( B \) is the noninactivating (pedestal) component of the current.

In addition to the inactivation time constant, the amplitude of the noninactivating pedestal component is expected to have a major influence on APD and hence APD restitution in cardiac myocytes. The component of \( Ca^{2+} \)-induced inactivation relative to the noninactivating pedestal component was strongly dependent on the subunit composition. \( Ca^{2+} \)-induced inactivation was most complete for \( \beta_{2b} \) (83 ± 11% of the total current) and \( \beta_{3} \) (84 ± 2%). The smallest fraction of \( Ca^{2+} \)-induced inactivation was observed with “no-beta” and \( \beta_{2a} \), such that only 55 ± 4 and 59 ± 0.3% of total \( Ca^{2+} \) current was inactivated by the 400-ms pulse, respectively. We have chosen this potential since the AP plateau in intact tissue is typically in the 10 to 20 mV range, as measured by glass microelectrode recordings (for example, see Ref. 25). Moreover, +10 mV is near the voltage at the end of AP plateau, which is most relevant to recovery from inactivation. These findings demonstrate that both the extent and time constant of \( Ca^{2+} \)-induced inactivation can be modulated differentially by altering the channel \( \beta \)-subunit composition. Note that the coexpression of \( \beta \)-subunits greatly increased current density. Recordings of \( \alpha_{1C} + \alpha_{2}\delta \) were performed 6–8 days after injection, whereas for all the other combinations with \( \beta \)-subunits 3–4 days were sufficient to achieve robust current density.
of 9–12 experiments. Ca\textsuperscript{2+} all subunit compositions displayed the bell-shaped relation, typical of a 1H9252 inactivation, which has not been previously characterized in 1H9252-subunit significantly slowed this fast component of the 1H11011 current. Recovery from inactivation was significantly larger: for \( \beta_{2a} \), \( \tau_{\text{fast}} = 112 \pm 10 \text{ ms} (75 \pm 2\%) \), \( \tau_{\text{slow}} = 2.018 \pm 201 \text{ ms} \); for \( \beta_{2b} \), \( \tau_{\text{fast}} = 108 \pm 7 \text{ ms} (82 \pm 15\%) \), \( \tau_{\text{slow}} = 1.830 \pm 13 \text{ ms} \); and for \( \beta_3 \), \( \tau_{\text{fast}} = 85 \pm 4 \text{ ms} (77 \pm 2\%) \), \( \tau_{\text{slow}} = 1.224 \pm 76 \text{ ms} \). These results show that the \( \beta \)-subunit composition of the L-type channels can differentially modulate the kinetics of recovery from inactivation.

Mathematical Modeling of Kinetic Changes Due to Subunit Modifications

To study how the \( \beta \)-subunit composition of the L-type Ca\textsuperscript{2+} channel is predicted to modulate the APD and the shape of the APD restitution curve, we simulated the effect of a given subunit modification on the kinetics of the L-type Ca\textsuperscript{2+} current as formulated in the Fox AP action potential model (3) modified to include our dynamic Calcium current (Ca\textsubscript{h}) cycling model (24). Our strategy is as follows: on the basis of the findings that the Ca\textsuperscript{2+} current in cardiac myocytes is most similar to \( \alpha_{1C} + \alpha_2\delta + \beta_{2b} \) channels (2), we simulated different subunit compositions by changing proportionately the kinetics of the L-type Ca\textsuperscript{2+} current in the Fox AP model by the same factor by which that subunit composition changed the \( \alpha_{1C} + \alpha_2\delta + \beta_{2b} \) current kinetics in the oocyte experiments. Thus, if \( \tau_{\text{myocyte}} \) is the time of inactivation of the Ca\textsuperscript{2+} current measured at physiological temperatures, as implemented in the Fox AP model, and if \( \tau_{\text{myocyte}}^{\beta} \) is that same time constant for the \( \beta_3 \) composition measured in our experiment, then

\[
\frac{\tau_{\text{myocyte}}}{\tau_{\text{myocyte}}^{\beta}} = \frac{\tau_{\text{myocyte}}}{\tau_{\text{myocyte}}^{\beta}}
\]

where \( \tau_{\text{myocyte}} \) and \( \tau_{\text{myocyte}}^{\beta} \) are found by estimating that same time constant directly from our current measurements of \( \alpha_{1C} + \alpha_2\delta + \beta_1 \) and \( \alpha_{1C} + \alpha_2\delta + \beta_{2b} \). Thus we can estimate the time constant of inactivation when \( \beta_3 \) is expressed in the cardiac myocyte as \( \tau_{\text{myocyte}}^{\beta_3} = \tau_{\text{myocyte}}^{\beta_3} \text{myocyte} \). In this way we could systematically evaluate how a given subunit modification should influence the kinetics of the L-type Ca\textsuperscript{2+} current under physiological conditions. Note that this approach assumes that the ratio of time constants, for different subunit compositions, is independent of temperature. A minor caveat is that the recovery from inactivation of the L-type Ca\textsuperscript{2+} current, as represented in the Fox model, only has one time constant of recovery, in contrast to our measurements of \( \alpha_{1C} + \alpha_2\delta + \beta_{2b} \). For this time constant we used the same time constants measured experimentally. The model parameters used are given in Table 2.

The predicted APs from the Shiferaw-Fox AP model with the three different \( \beta \)-subunit compositions incorporated into the L-type Ca\textsuperscript{2+} current model are shown in Fig. 5A. The long dashed line corresponds to the AP generated by using the WT L-type Ca\textsuperscript{2+} current parameters at steady state (1-s cycle length pacing). All the kinetic modifications of the native currents tended to increase APD, with a marked APD prolongation for the \( \beta_{2a} \) and \( \alpha_{1C} + \alpha_2\delta \) compositions and only a modest increase for \( \beta_3 \). The corresponding Ca\textsuperscript{2+} transients (Fig. 5B) show that the \( \beta_{2a} \) and \( \alpha_{1C} + \alpha_2\delta \) compositions also lead to Ca\textsuperscript{2+} concentrations levels that are higher than in control, a direct consequence of the increase of Ca\textsuperscript{2+} entry due to the incomplete inactivation.

Recovery from Inactivation of L-type Ca\textsuperscript{2+} Current

To investigate the effects of \( \beta \)-subunits on recovery from inactivation, which has not been previously characterized in detail for \( \beta \)-subunits with Ca\textsuperscript{2+} as the charge carrier, we used a two-pulse protocol. From a holding potential of \(-90 \text{ mV} \), an inactivating (1-s) pulse to \(+10 \text{ mV} \) (P1) was followed, after a variable duration of repolarization, by a short (100-ms) test pulse (P2) of the same amplitude. The time interval between P1 and P2 was progressively increased to monitor channel recovery. The interval between every repetitive cycle (between P2 and P1) was 20 s to avoid accumulation of inactivation. Representative experiments for the different subunit composition are shown in Fig. 4, A–D. The corresponding time course of the recovery from inactivation is shown in Fig. 4, E–H. The recovery kinetics were well fit by a biexponential function (fit superimposed, solid line). The averages of the fitting parameters for the fast and slow components of recovery are reported in Table 1. The fastest time constant of recovery from inactivation (37.8 \pm 3 ms) was observed for the \( \alpha_{1C} + \alpha_2\delta \) combination. However, this component only accounted for \( \sim 53\% \) of the total current recovery. Coexpression of any \( \beta \)-subunit significantly slowed this fast component of the recovery, and the fraction of the current undergoing faster recovery from inactivation was significantly larger. Data are averages of 9–12 experiments \( \pm \) SE. The rate of inactivation vs. membrane potential for all subunit compositions displayed the bell-shaped relation, typical of a Ca\textsuperscript{2+}-dependent process.

![Graph](https://example.com/graph.png)
induced by these modifications. Thus we have adjusted the maximum conductance of the L-type Ca\(_{\text{2+}}\) current so that all subunit compositions had a similar APD at a DI of 350 ms. In this way, the influence of subunit expression on restitution kinetics can be evaluated while maintaining a comparable APD and Ca\(_{\text{i}}\) transient magnitude. Figure 5C shows the S1-S2 APD restitution curves for the WT cardiac myocyte (long dashed line) and the corresponding curves for the various β-subunit modifications. APD restitution was substantially modified in the β\(_{2a}\) and α\(_{1C}\) + α\(_{2D}\) compositions. Figure SD shows the slopes of APD restitution measured by the S1-S2 interval pacing protocol in the model (see MATERIALS AND METHODS). The control APD restitution

Table 1. Averages of the fitted fast and slow time constants of recovery from inactivation and their relative amplitudes for the different subunit compositions

<table>
<thead>
<tr>
<th>Subunit Composition</th>
<th>α(<em>{1C}) + α(</em>{2D})</th>
<th>α(<em>{1C}) + α(</em>{2D}) + β(_{1})</th>
<th>α(<em>{1C}) + α(</em>{2D}) + β(_{2a})</th>
<th>α(<em>{1C}) + α(</em>{2D}) + β(_{3})</th>
<th>τ(_{\text{fast}}), ms</th>
<th>τ(_{\text{slow}}), ms</th>
<th>Amp(_{\text{fast}}), %</th>
<th>Amp(_{\text{slow}}), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte (WT)</td>
<td>37.8±3</td>
<td>85±4</td>
<td>112±10</td>
<td>108±7</td>
<td>422.4±18</td>
<td>1,224±76</td>
<td>2,018±201</td>
<td>1,830±13</td>
</tr>
<tr>
<td>α + α(<em>{2D}) + β(</em>{3})</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
</tr>
</tbody>
</table>

Values are averages ± SE. τ\(_{\text{fast}}\) and τ\(_{\text{slow}}\), fast and slow time constants of recovery from inactivation, respectively. Amp\(_{\text{fast}}\) and Amp\(_{\text{slow}}\), fast and slow amplitudes, respectively.

Table 2. L-type Ca\(_{\text{2+}}\) current parameters as formulated in the Fox AP model, and the corresponding β-subunit modifications

<table>
<thead>
<tr>
<th>w</th>
<th>f(_{0})</th>
<th>τ(_{\text{m}}), ms</th>
<th>t(_{\text{in}}), ms</th>
<th>t(_{\text{sout}}), ms</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte (WT)</td>
<td>1</td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>α + α(<em>{2D}) + β(</em>{3})</td>
<td>0.91</td>
<td>0.29</td>
<td>41</td>
<td>41.6</td>
<td>2.018</td>
</tr>
<tr>
<td>α + α(<em>{2D}) + β(</em>{2a})</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>α + α(<em>{2D}) + β(</em>{2b})</td>
<td>0.65</td>
<td>0.33</td>
<td>29.4</td>
<td>32.1</td>
<td>122.4</td>
</tr>
</tbody>
</table>

The last column (% reduction) indicates the percent current reduction in order to normalize AP durations as shown in Fig. 5B. AP, action potential; WT, wildtype; w, weight factor; f\(_{0}\), constant that fixes the minimum steady-state value of the voltage- and Ca\(_{\text{2+}}\)-dependent inactivation gate; τ\(_{\text{m}}\), inactivation time constant; t\(_{\text{in}}\) and t\(_{\text{sout}}\), fast and slow recovery time constants, respectively.
The slope was relatively steep (>1) at short DI, whereas the slopes for αIC + αδ and β2a were shallower (<1) over all DI values. The corresponding APs and Ca2+ transients during steady-state pacing at 400 ms are shown in Figure 5, E and F. Note that since the cycle length is faster, the APD and Ca2+ transient are modified. To evaluate the effect of decreasing the conductance of the Ca2+ current, independently of a concomitant modification of the inactivation kinetics, we have also simulated the effect of a general Ca2+ channel blocker such as verapamil. Here, we simulate the blocker effect by decreasing the conductance of the Ca2+ current by 50%. In Fig. 5, E and F, we show the AP and Ca2+ transient in this case (dotted line), indicating that direct Ca2+ current blockage leads to a dramatic decrease of both APD and peak Ca2+.

**Effect of Subunit Modifications on Spiral Wave Dynamics**

To characterize the effects of the changes in APD restitution produced by various subunit compositions on the stability of reentry, we initiated reentry using a cross-field stimulation in simulated two-dimensional tissue (6.75 × 6.75 cm2). Figure 6A shows voltage snapshots at 400 and 800 ms using the Shiferaw-Fox AP model, which had steep S1-S2 restitution (dashed black line in Fig. 5B). As shown, a spiral wave that was initiated in the tissue was unstable and broke up into several wave fronts typical of a VF-like state. This breakup occurred after four spiral wave rotations. The spiral wave meandered in a chaotic fashion and as the wave tip followed the wave back. After 2 s, the fractionated wave fronts self-terminated by meandering to and colliding with borders of the tissue. Figure 6B shows the effects of the αIC + αδ-subunit composition, which had the greatest effect at flattening APD restitution slope. In this case the initiated spiral wave remained intact as a single reentrant wave rotating around a stable circular core and did not break up into VF-like state over the full 3-s duration (20 spiral wave rotations) of the simulation. Similarly, Fig. 6C shows the spiral wave evolution at 600 ms and 3 s for the β2a-subunit parameters, again showing that the spiral wave was stabilized. It is interesting to note that the spiral wave tip morphology was different for each subunit composition tested. For the αIC + αδ-subunit composition, the wavelength of the spiral wave was shortened at short DI, which led to an increase in the excitable area near the spiral tip. This effect tended to
stabilize the spiral wave as the propensity for wave break was decreased. On the other hand, the $\beta_{2a}$ case led to a blunted spiral tip with a larger wavelength. However, in this case the wavebreak was smooth and did not induce wave break.

**DISCUSSION**

We have characterized the inactivation and recovery kinetics of $\alpha_{1c} + \alpha_2\delta$ channels (Ca.1.2) expressed alone ($\alpha_{1c} + \alpha_2\delta$) and with $\beta_1$, $\beta_{2a}$, and $\beta_{2b}$-subunits expressed in oocytes, using Ca$^{2+}$ as the charge carrier. The rate of inactivation at $+10\text{ mV}$, relevant to the late plateau phase of the cardiac AP that determines APD, was slowest in the $\alpha_{1c} + \alpha_2\delta$ channels and progressively accelerated in the order $\alpha_{1c} + \alpha_2\delta < \beta_1 < \beta_{2a} < \beta_{2b}$. The amplitude of the Ca$^{2+}$-induced inactivation component strongly depended on subunit composition. The inactivating component of Ca$^{2+}$ current mediated by channels expressed without $\beta$ and channels coexpressed with $\beta_{2a}$ was 55 and 59%, respectively, whereas coexpression of $\beta_{2b}$ and $\beta_3$ increased the inactivation to 83–84% of the total current. These differences had profound effects on the simulated ventricular APD, APD restitution, and spiral wave dynamics.

Whereas APD is sensitive to both the time constant and extent of Cu$^{2+}$-induced inactivation, APD restitution at short DI primarily depends on the rate of L-type Ca$^{2+}$ current recovery from inactivation. In oocytes, we found that the time course of recovery was biexponential. For all the $\beta$-subunits studied, the fast component of recovery from inactivation had a time constant in the 100-ms range, whereas the slower component had a time constant in the 1- to 2-s range. A double exponential time course of recovery from inactivation has also been described in human ventricular myocytes (9). The amplitudes of the recovery components were similar for all the $\beta$-subunit compositions we tested, with the fast component accounting for $\sim 75$–80% of the total current recovery. However, in the absence of any $\beta$-subunit the channel recovery kinetics were significantly different. In this case, the fast and slow components each accounted for $\sim 50\%$ of recovery, and both time constants were accelerated by a factor of $\sim 2$, compared with when a $\beta$-subunit was present. These results demonstrate that the recovery kinetics of the L-type Ca$^{2+}$ current were relatively insensitive to $\beta$-subunits but changed dramatically when $\beta$-subunits were eliminated.

To predict the physiological effects of different subunit compositions of the L-type Ca$^{2+}$ channel on the cardiac AP and APD restitution properties, we incorporated the measured kinetic properties of the L-type Ca$^{2+}$ current into a detailed ventricular AP model. To evaluate the effect of subunit composition changes on AP properties, we rescaled the WT Ca$^{2+}$ current kinetics, using $\alpha_{1c} + \alpha_2\delta + \beta_{2a}$ as reference. Implementing these kinetic changes, we found that all subunit modifications increased the pedestal current of the L-type Ca$^{2+}$ current, which, in the model, increased net inward current during the AP plateau. In particular, the coexpression with $\beta_{2a}$ and $\alpha_{1c} + \alpha_2\delta$ modifications prolonged APD dramatically to $>500\text{ ms}$, which may be proarrhythmic if not corrected by shortening APD back to control levels. Indeed, a defective L-type Ca$^{2+}$ channel inactivation is considered the underlying mechanism in the Timothy Syndrome, a congenital form of long QT (28). Thus this study also emphasizes that to take advantage of the potency of the kinetic effects derived by the subunit modifications in preventing wave break, a control of the total Ca$^{2+}$ entry has to be taken into consideration to prevent proarrhythmic consequences of APD prolongation and intracellular Ca$^{2+}$ overload. To study the effects on APD restitution and spiral wave stability in the model, we made the latter adjustment for all of the modified subunit compositions by reducing the maximum conductance of the L-type Ca$^{2+}$ current sufficiently to return APD back to its control value near $\sim 250\text{ ms}$. As shown in Fig. 5C, APD restitution slope for the native L-type Ca$^{2+}$ current was relatively steep (slope $>1$) at short DI, leading to spiral wave breakup into a VF-like state in two-dimensional tissue, as shown in Fig. 6A. Both of these properties, APD restitution slope $>1$ and spontaneous spiral wave breakup, are characteristic features of ventricular muscle from many mammalian species, including humans (7, 30, 36). All other subunit combinations exhibited flatter APD restitution slopes. In $\beta_3$, the effect was only modest and not sufficient to flatten APD restitution slope to $<1$. On the other hand, the $\beta_{2a}$ and $\alpha_{1c} + \alpha_2\delta$ cases flattened APD restitution slope to $<1$.

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**Fig. 6. Effect of subunit composition on reentrant spiral wave dynamics in simulated 2D tissue.** A: voltage snapshots at 400 and 800 ms after the initiation of the spiral wave, for the WT L-type Ca$^{2+}$ current as formulated in the Shiferaw-Fox AP model. The spiral wave broke up after 5 rotations into multiple wavelets, before spontaneously terminating after 2 s. Note that initial conditions in the tissue were the same as after 1-s pacing to steady state. B: voltage snapshots after 600 ms and 3 s (20 rotations) after the initiation of the spiral wave by using the $\alpha_{1c} + \alpha_2\delta$-subunit modification. Recently remained stable without spiral wave breakup. C: same as above using the $\beta_{2a}$ composition parameters. As shown, reentry remained stable for up to 3 s (20 rotations).
everywhere. So both of these subunit modifications prevented spiral wave breakup, as demonstrated in Fig. 6, B and C.

Our simulation results showed that both the $\beta_{2a}$ and $\alpha_{1C} + \alpha_{2}\delta$ compositions, along with a reduction of $Ca^{2+}$ current amplitude, led to a flattened APD restitution curve. To understand the underlying mechanism for this effect, in Fig. 7A we have plotted the $Ca^{2+}$ current during the S1-S2 protocol used to compute the restitution curves shown in Fig. 5C. Families of superimposed $Ca^{2+}$ currents for the last S1 beat and for a wide range of S2 intervals are shown for both the WT and the $\alpha_{1C} + \alpha_{2}\delta$ composition. As expected the WT $Ca^{2+}$ current completely inactivates and subsequently recovers from a small peak value ($-10 \, pA/pF$) to a significantly higher value ($-30 \, pA/pF$) as the S1-S2 interval is increased. On the other hand, the $\alpha_{1C} + \alpha_{2}\delta$ composition recovers from a current amplitude of $-10 \, pA/pF$ to about $-15 \, pA/pF$, since lesser inactivation occurred in the last S1 beat. To confirm that $Ca^{2+}$ current entry indeed dictates APD restitution, in Fig. 7B we plot the integrated $Ca^{2+}$ current elicited by S2 beat as a function of DI. Indeed, the integral of the $Ca^{2+}$ current closely resembles the APD restitution curve (for WT and $\alpha_{1C} + \alpha_{2}\delta$) shown in Fig. 5C. Thus in this case the flattening of the restitution is directly related to the fact that recovery from inactivation is diminished since the overall extent of inactivation was reduced.

To confirm that the flattening of the restitution curve is due primarily to the change in current kinetics and not to the reduction in the $Ca^{2+}$ current amplitude, we have simulated the restitution properties for the $\alpha_{1C} + \alpha_{2}\delta$ composition when the current conductance is the same as that for the WT. In this case, the APD becomes unphysiologically large ($\sim 800 \, ms$) owing to
the larger pedestal current. To reduce the APD to control levels (~250 ms) we have increased the time-independent plateau potassium current ($I_{Kp}$) in the Fox model by a factor of 8. In Fig. 7C we plot the S1-S2 restitution curve after pacing at a cycle length of 400 ms for 20 beats using both the WT and the $\alpha_{1C} + \alpha_2\delta$ composition. As shown, in Fig. 7, C and D, the $\alpha_{1C} + \alpha_2\delta$ restitution curve is significantly flattened. In this case the flattening of the restitution curve is independent of changes in the Ca$^{2+}$ current conductance since it has been maintained identical for both simulations. Thus the kinetic changes induced by the subunit composition changes will robustly flatten the restitution curve.

An interesting finding of this study is that the modulation of Ca$^{2+}$ current kinetics seems to be potentially applicable for therapy only if the subunit changes are implemented in conjunction with a reduction of the current amplitude. For both the $\beta_2a$ and $\alpha_{1C} + \alpha_2\delta$ compositions, inactivation of the Ca$^{2+}$ current was dramatically altered so that the APD and Ca$^{2+}$ current entry was increased to unphysiological levels. However, we found that if the current amplitude was reduced, as for instance when a Ca$^{2+}$ channel blocker such as verapamil is applied, it is then possible both to flatten APD restitution and to maintain physiological Ca$^{2+}$ levels. In effect, the Ca$^{2+}$ entry due to the larger pedestal current allows the current amplitude to be decreased, which in effect reduces the contribution of Ca$^{2+}$ current kinetics and also returns the Ca$^{2+}$ transient back to physiological levels (Fig. 5, E and F). Thus, in this case the detrimental effects of the subunit composition changes could be counteracted by simply decreasing the current amplitude. These results highlight the complex interplay of various components of the cell electrophysiology, so that targeting the ion channel kinetics leads to downstream changes that must be balanced by at least one more independent change.

Some studies (8) have argued that the dynamic rather than S1-S2 restitution curve is predictive of spiral wave breakup. The dynamic restitution curve is found by measuring the steady-state APD as a function DI at a fixed pacing cycle length. Since this is a steady-state measurement, the dynamic restitution is sensitive to a variety of slow ionic processes, such as Na$^+$ and Ca$^{2+}$ ion accumulation regulated by ion exchangers and pumps. Consequently, the Shiferaw-Fox model using the WT parameters reached steady state only after about 20 beats. Since this time is much longer than the roughly three to five spiral wave rotations needed for an intact spiral wave to break up, the dynamic restitution, in this case, is less directly relevant to spiral wave stability. On the other hand, the S1-S2 restitution curve reflects the immediate response of the APD to variations in DI induced by a change in cycle length. Hence a steep S1-S2 restitution slope leads to large APD variations along the spiral wave, which leads to a nonuniform waveback distribution that induces wave break. Indeed, this is consistent with our simulation results since the $\beta_2a$ and $\alpha_{1C} + \alpha_2\delta$ compositions directly influenced Ca$^{2+}$ current recovery and thus S1-S2 restitution slope.

It is important to stress that the approach presented here is distinct from previous studies (4, 23) showing that marked reduction of the L-type current, via a Ca$^{2+}$ channel blocker such as verapamil, can flatten restitution slope and prevent spiral wave breakup. In those studies, the reduction of the L-type current led to a large decrease in Ca$^{2+}$ entry and thus markedly suppressed contractility. In our numerical study, Ca$^{2+}$ entry is preserved since the reduction of the L-type current is introduced only to compensate for the overall reduced inactivation observed with the “$\beta_2a$” and $\alpha_{1C} + \alpha_2\delta$ composition. Therefore, flattening of APD restitution is achieved while preserving normal and preventing excessive Ca$^{2+}$ entry into the cell. In Fig. 5, E and F, we simulated the effect of a direct decrease of the Ca$^{2+}$ current conductance (50% reduction) and indeed found that the peak Ca$^{2+}$ transient is reduced by almost a factor of 5, whereas the APD is decreased by roughly a factor of 2. The dramatic decrease in peak Ca$^{2+}$ occurs over several paced beats, since a new equilibrium point must be reached, in which the efflux due to the Na/Ca exchanger current will balance the current influx due to the reduced Ca$^{2+}$ current. In general, this leads to a marked reduction of the sarcoplasmic reticulum content since Ca$^{2+}$ influx is essentially cut in half. The advantage of decreasing the expression levels of $\beta_3$ and $\beta_{2a}$ or overexpressing $\beta_{2a}$ along with a reduction in the current conductance is that Ca$^{2+}$ entry over one beat can be preserved. Thus control of $\beta$-subunit expression offers an alternative way of controlling restitution slope and wave stability.

Given the results in this study, it is worth asking whether Ca$^{2+}$ channel subunit modifications can be developed to the point of being a viable therapeutic strategy. A particular limitation of current gene therapy approaches is that expression levels tend to be heterogeneous. However, it is important to point out that electrotonic coupling dramatically smooths out cell-to-cell heterogeneities. Thus, the challenge is to modify “enough” cardiac cells to change the electrophysiology of cardiac tissue over a tissue scale that is relevant to the dynamics of a spiral wave. With improved vector technology, this may be achievable in the foreseeable future.

**Limitations**

One important limitation predicted by this study is that the Ca$^{2+}$ channel subunit modifications yield therapeutic effects only if the Ca$^{2+}$ current amplitude is adjusted to keep transsarcolemmal Ca$^{2+}$ entry roughly constant in the face of different inactivation kinetics. This is essential for avoiding either excessive suppression of the Ca$^{2+}$ transient or Ca$^{2+}$ overload, which can be directly arrhythmogenic. The Ca$^{2+}$ current amplitude is, however, a tractable target, since different Ca$^{2+}$ current blockers are available and can be dosed to achieve a desired level of current block. In the broader perspective, it is likely that any therapeutic strategy that targets current kinetics will likely inflict side effects that will have to be controlled by an independent mechanism. This issue certainly deserves careful attention because it is likely to complicate any therapeutic proposal to modulate cell electrophysiology.

Intracellular Ca$^{2+}$ cycling is another important dynamic factor influencing wave stability in addition to APD restitution (33), and the L-type Ca$^{2+}$ current has a major influence on intracellular Ca$^{2+}$ cycling dynamics. Although the Shiferaw-Fox model contains dynamically active Ca$^{2+}$ cycling, the effects of the L-type Ca$^{2+}$ current modifications simulated in this study did not exacerbate Ca$^{2+}$-cycling-mediated wave instabilities to the point that they interfered with the ability of flattening of APD restitution slope to prevent spiral wave breakup. However, this issue needs to be studied in more detail. Finally, the kinetic parameters of reconstituted L-type Ca$^{2+}$ channels in oocytes may not be the same as that
as in cardiac myocytes. A mammalian cell line studied at 37°C under perforated patch-clamp conditions would be preferable for characterizing different subunit compositions. Nevertheless, our results represent a starting point for further exploration of gene-based therapeutic strategies in mammalian hearts.

With recognition of the limitations discussed above, these results offer a first proof of concept that a nonpharmacological tool (i.e., the use of modulatory subunit specific for Ca²⁺ channels) might be strategically applied to control cardiac wave stability to prevent spiral wave breakup in the heart.

ACKNOWLEDGMENTS

We thank Nikolai Soldatov for constructive discussions and providing the human α₁C clone and Ning Qin for providing the α₂β₃- and β-subunits.

GRANTS

This research was supported by National Institutes of Health Grants R01 NS-043240, P50 HL-53219, and P01 HL-078931 and by Laubisch and Kawata Endowments.

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