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Modulation of action potential by $[Ca^{2+}]_i$ in modeled rat atrial and guinea pig ventricular myocytes

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Han, Chunlei, Pasi Tavi, and Matti Weckström. Modulation of action potential by $[Ca^{2+}]_i$ in modeled rat atrial and guinea pig ventricular myocytes. *Am J Physiol Heart Circ Physiol* 282: H1047–H1054, 2002; 10.1152/ajpheart.00573.2001.—We simulated mechanisms that increase Ca^{2+} transients with two models: the Luo-Rudy II model for guinea pig (GP) ventricle (GP model) representing long action potential (AP) myocytes and the rat atrial (RA) model exemplifying myocytes with short APs. The interventions were activation of stretch-gated cationic channels, increase of intracellular Na^+ concentration ($[Na^+]_i$), simulated β -adrenoceptor stimulation, and Ca^{2+} accumulation into the sarcoplasmic reticulum (SR). In the RA model, interventions caused an increase of AP duration. In the GP model, AP duration decreased except in the simulated β -stimulation where it lengthened APs as in the RA model. We conclude that the changes in the APs are significantly contributed by the increase of the Ca^{2+} transient itself. The AP duration is controlled differently in cardiac myocytes with short and long AP durations. With short APs, an increase of the Ca^{2+} transient promotes an inward current via Na^+/Ca^{2+} -exchanger lengthening the AP. This effect is similar regardless of the mechanism causing the increase of the Ca^{2+} transient. With long APs the Ca^{2+} transient increase decreases the AP duration via inactivation of the L-type Ca^{2+} current. However, L-type current increase (as with β -stimulation) increases the AP duration despite the simultaneous Ca^{2+} transient augmentation. The results explain the dispersion of AP changes in myocytes with short and long APs during interventions increasing the Ca^{2+} transients.

heart; cardiac; calcium; ion channels; contraction

THE LENGTH AND SHAPE OF action potentials (APs) of cardiac myocytes are determined by concerted activation and inactivation of depolarizing and repolarizing currents during excitation. Depolarization is controlled primarily by Na^+ and Ca^{2+} currents, whereas repolarization results mostly from activation of several K^+ currents (3, 7). During the AP repolarization phase, several currents are also influenced by Ca^{2+} released from the sarcoplasmic reticulum (SR). Therefore, any stimuli affecting the systolic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) will also influence the AP (19, 38). However, the impact of $[Ca^{2+}]_i$ on the AP shape de-

pends on the relative contribution of the Ca^{2+} -dependent currents on the repolarization of the given myocytes. If $[Ca^{2+}]_i$ -dependent regulation of the AP depends strongly on the length of the AP, the increase of the Ca^{2+} transients would result in a different effect on the APs with short or long duration.

In cardiac myocytes Ca^{2+} transient augmentation can be caused by many mechanisms that are utilized by several hormones and also mechanical stretch of the myocyte. The most powerful stimuli to produce Ca^{2+} transient augmentation are the activation of β -adrenoceptors. Activation of these receptors leads to liberation of cAMP and subsequent activation of protein kinase A (PKA), which phosphorylates L-type Ca^{2+} channels and phospholamban, the protein regulating the SR Ca^{2+} -ATPase, the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA). In addition to direct regulation of Ca^{2+} handling proteins Ca^{2+} transient also can be augmented indirectly. The mechanism of endothelin-1 (ET-1) in the cardiac myocytes involves activation of protein kinase C (PKC). The PKC-induced phosphorylation of the Na^+/H^+ exchanger promotes an increase of the intracellular Na^+ concentration ($[Na^+]_i$), thereby activating the Na^+/Ca^{2+} exchanger and thus promoting Ca^{2+} influx and subsequent augmentation of the Ca^{2+} transients (1). Activation of cation-selective stretch-activated (SA) ion channels would also result in sodium accumulation with similar events that followed.

Because of the interrelationship between APs and intracellular free Ca^{2+} , all mechanisms able to increase Ca^{2+} transients would have a distinct impact on the shape of APs, and this would conceivably depend on the initial length of the AP. Because the contribution of Ca^{2+} currents and the Na^+/Ca^{2+} exchanger current ($I_{Na/Ca}$) on the AP repolarization depends on the length of the AP (8, 19, 38), it is possible that activation of the same Ca^{2+} pathway can produce different AP change in different types of myocytes. To test this hypothesis, we simulated the effect of known mechanisms producing positive inotropy with two mathematical models: the Luo-Rudy II model for guinea pig (GP) ventricles representing long AP myocytes (GP model), and the rat

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atrial (RA) model as an example of myocytes with short APs (27). When designing the inotropic interventions to be used in the models, one boundary condition was that the effects on the Ca^{2+} transients should be modest and approximately of the same size, regardless of the type of intervention. This makes it easier to compare the different mechanisms to each other, which facilitates understanding of the regulation of the AP length.

MATERIALS AND METHODS

Mathematical Models

The Luo-Rudy II model is a GP model that describes the membrane ionic currents, $[\text{Ca}^{2+}]_i$, and sarcoplasmic reticular (SR) function dynamically. It was developed by Luo and Rudy in 1994 (20), and it has been used successfully to simulate the early afterdepolarization (35), delayed afterdepolarization, acute myocardial ischemia (24), the role of ATP-sensitive K^+ current (9), and slow changes in response to step change in length (5). The development of this model is mainly based on guinea pig ventricular (GP) myocytes, which have a long AP [the AP duration at the 90% level (APD_{90}) is ~ 150 ms]. In the present study, this GP model was employed as a representative of species with long APD.

To represent myocytes with short APs ($\text{APD}_{90} \sim 50$ ms) we used our previously published RA model (27). The major differences of this model and the GP model are in the potassium currents as detailed previously (27), and all the Ca^{2+} -handling pathways are modeled similarly.

All simulations at 1 Hz were run as long as a steady state of all parameters of the model were reached, normally ~ 10 min. The effect of any simulated intervention was adjusted in the affected model parameters, on the basis of experimental results, with the increase of the Ca^{2+} transient amplitude (15–30%). Comparisons were done between the control and the endpoint (steady state) of the simulation with different simulation strategies. All models were created with the use of a Sun workstation running MATLAB version 5.3 (Mathworks).

Simulation Protocols

SA ion channels. The stretch-mediated changes were explored in both the RA and GP models by implementing SA conductance, and the ensuing current (I_{stretch}) into the model cell membrane. The properties of this conductance were based on the features of the SA channels in the experimental findings from isolated rat ventricular myocytes (36). I_{stretch} is a nonselective cationic current, carrying Na^+ and K^+ ions with a reversal potential of -6 mV (37) with the following formalism

$$I_{\text{stretch}} = -\gamma_{\text{Na}}(8.3 - 5\text{SL})(E_m - E_{\text{Na}}) - \gamma_{\text{K}}(8.3 - 5\text{SL})(E_m - E_{\text{K}})$$

where γ = maximal conductance, $\gamma_{\text{Na}} = 0.9$ nS, $\gamma_{\text{K}} = 1.17$ nS, SL is sarcomere length, E_m is membrane potential, E_{Na} is sodium reversal potential, and E_{K} is potassium reversal potential. The SL of $1.75 \mu\text{m}$ is set as a resting length of sarcomere. In stretch simulation, the length of sarcomere is increased 3.2%, i.e., to equivalent of $1.8 \mu\text{m}$ (see also Ref. 27).

Simulation of the ET-1-induced Ca^{2+} transient augmentation. During ET-1 stimulation $[\text{Na}^+]_i$ increases because of the activation of the Na^+/H^+ exchanger via PKC phosphorylation. This leads to a secondary increase in the $[\text{Ca}^{2+}]_i$ transients (1). Because Na^+ accumulation into the cytosol is the change that promotes Ca^{2+} accumulation, we simulated the

ET-1 effect by increasing $[\text{Na}^+]_i$ from 10 to 14 mmol/l in both models.

Ca^{2+} accumulation by PKA phosphorylation. The increase of Ca^{2+} transients during β -adrenoceptor stimulation results from the PKA-induced phosphorylation of L-type Ca^{2+} channels and the phospholamban. The L-type channel phosphorylation increases the peak Ca^{2+} current during excitation. The mechanism of this may be the shift of the current-voltage relation towards more negative potentials (14), resulting in larger Ca^{2+} current during APs. The simultaneous phospholamban phosphorylation increases Ca^{2+} storage into the SR stores via increased activity of SERCA, leading to augmented Ca^{2+} release during Ca^{2+} -induced Ca^{2+} release. To simulate modest increase of the Ca^{2+} transients during β -stimulation the maximum conductance of the L-type Ca^{2+} channel was increased by 1.5, and the the SERCA activity was increased by 1.5. In strong β -stimulation, the changes in the Ca^{2+} transients are much bigger (see Ref. 3 for a review) but in this study this minor change was used to facilitate comparison with the results of other inotropic interventions in the model.

SR Ca^{2+} content. To simulate the SR Ca^{2+} loading, we had to take into account the different contribution of the SR Ca^{2+} release in the two different types of myocytes. In the guinea pig ventricle, the contribution of the SR Ca^{2+} release on the Ca^{2+} transient is ~ 60 –70%, and this ratio is much greater, up to 93%, in the rat atrium (4). To produce an equal change in the Ca^{2+} transient in both models we increased the SR Ca^{2+} content by 20% in the RA model and by 50% in the GP model. Again, this does not necessarily reflect the true relationship of the effects of SR loading in the two cell types but produced changes in the Ca^{2+} transients that could easily be compared with other interventions.

Simulated clamping of $I_{\text{Ca,L}}$ or $I_{\text{Na/Ca}}$. For exploring the different contributions of the L-type Ca^+ current ($I_{\text{Ca,L}}$) and the $I_{\text{Na/Ca}}$ on the APD in response to elevation of $[\text{Ca}^{2+}]_i$, we used simulations with “clamped” L-type currents and $I_{\text{Na/Ca}}$. That means that under conditions of increased Ca^{2+} transients, induced by increased SR Ca^{2+} content, the time-course of the Ca^{2+} transient was clamped to the value during the intervention, and either $I_{\text{Ca,L}}$ or $I_{\text{Na/Ca}}$ was forced (“clamped”) to the value in control condition. All other variables were allowed to change freely. In this way, it was possible to isolate directly the contribution of $I_{\text{Ca,L}}$ or $I_{\text{Na/Ca}}$ to the change of APD.

RESULTS

The simulations presented in this study investigate the effects of Ca^{2+} transient augmentation on the AP waveform of the cardiac myocytes. By employing two mathematical models of cardiac myocytes with different electrophysiological characteristics, we examined the potential mechanisms causing the diversity of changes of APDs induced by different strategies of Ca^{2+} -induced inotropy in short and long AP species. Simulations included the following: 1) activation of SA-channel current (I_{stretch}), 2) elevation of the $[\text{Na}^+]_i$ to promote Ca^{2+} influx through reverse mode of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Na_i^+), 3) increase of the $I_{\text{Ca,L}}$ during and increased activity of SERCA, and 4) increased Ca^{2+} accumulation into the SR. The main results from the present study are summarized in numerical form in Table 1, where parameters for AP configuration (APD_{30} and APD_{90}), the main Ca^{2+} -modulated membrane currents (the peaks of $I_{\text{Ca,L}}$ and

Table 1. Results for important indexes of APs, $I_{Ca,L}$, $I_{Na/Ca}$, and intracellular sodium under control and different stimulation conditions

	APD ₃₀ , ms	APD ₉₀ , ms	Peak $[Ca^{2+}]_i$, μM	Peak $I_{Ca,L}$, $\mu A/\mu F$	$rI_{Na/Ca}$, $\mu A/\mu F$	$iI_{Na/Ca}$, $\mu A/\mu F$	Na^+_i , mM
Rat							
Control	6.50	50.1	1.02	-7.94	2.59	-1.86	10.8
SR Ca^{2+}	5.49	64.9	1.92	-8.79	3.66	-2.22	12.2
$I_{stretch}$	5.86	60.3	1.48	-8.39	3.13	-2.06	11.5
Na^+_i	5.78	54.6	1.47	-8.73	3.54	-2.14	12.0
$I_{Ca,L}/SERCA$	6.27	64.0	2.12	-10.94	2.63	-1.98	10.8
Guinea pig							
Control	70.2	143	1.17	-5.00	1.01	-1.07	11.1
SR Ca^{2+}	36.3	109	1.92	-5.96	1.70	-1.52	13.4
$I_{stretch}$	54.7	118	1.33	-6.29	1.61	-1.14	13.1
Na^+_i	52.7	114	1.39	-6.33	1.95	-1.27	14.0
$I_{Ca,L}/SERCA$	65.2	172	2.10	-8.25	1.01	-1.02	10.8

AP, action potential; $I_{Na/Ca}$, Na^+/Ca^{2+} exchanger current; APD₃₀ and APD₉₀, duration of AP at 30% and 90% repolarization; $rI_{Na/Ca}$, peak of the reverse $I_{Na/Ca}$; $iI_{Na/Ca}$, peak inward $I_{Na/Ca}$. Na^+_i , intracellular ion concentrations; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; SR Ca^{2+} , Ca^{2+} accumulation into the sarcoplasmic reticulum; $I_{stretch}$, stretch-activated conductance and current; $I_{Ca,L}$, L-type Ca^{2+} current; SERCA, Sarc(endo)plasmic reticulum Ca^{2+} -ATPase.

$I_{Na/Ca}$), and two main intracellular ion concentrations ($[Ca^{2+}]_i$ and $[Na^+]_i$) are given in all simulation protocols in RA and GP model cells.

Activation of SA Ion Channels

Figure 1 shows the effect of the activation of a non-selective SA conductance in RA and GP models. The peak $[Ca^{2+}]_i$ transient was increased to 1.48 μM from 1.02 μM in RA model (Fig. 1A) and to 1.33 μM from 1.16 μM in GP model cell (Fig. 1B). The duration of the AP is prolonged in RA model cell and decreased in GP model. APD₉₀ increased from 52 ms to 61 ms in RA model, and decreased from 144 to 129 ms in the GP model, respectively. Because the reversal potential of the SA current (I_{SAC}) was -6 mV in the model, it will generate an inward (depolarizing) current at voltage values below this value of membrane voltage. How-

ever, at more positive potentials I_{SAC} is outward, and will accelerate the early repolarization in both model APs. This effect is small in the RA model but more prominent in the GP model. The other contributing factor to the change in APs is the increased Ca^{2+} release. Because the SA current is mainly carried by Na^+ ions, the activation of the current promotes Na^+ accumulation, which is compensated by the changes in the $I_{Na/Ca}$. SA channel current therefore causes increased Ca^{2+} accumulation and its increased storage into the SR, leading to augmented Ca^{2+} release during excitation. In the GP model, the increased Ca^{2+} transient causes more prominent Ca^{2+} -dependent inactivation of the $I_{Ca,L}$, which as such shortens the AP duration. The peak of the $I_{Ca,L}$ is slightly larger because of the small decrease in voltage-dependent inactivation due to the small decrease in the peak depolarization of

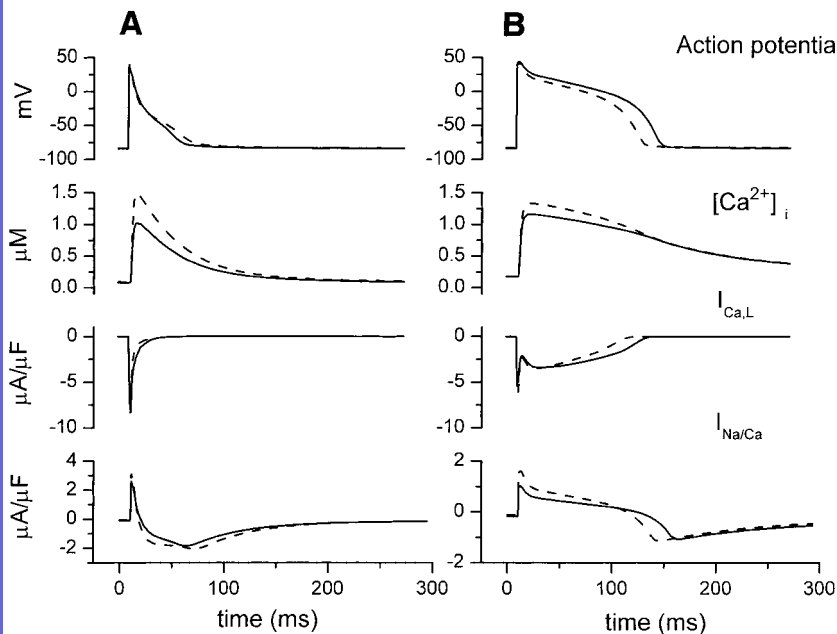


Fig. 1. Effects of the stretch-activated (SA) current (I_{SAC}) on model cells. Comparison of steady-state responses before (solid line) and after (dashed line) stretch. A: rat atrial (RA) model; B: Luo-Rudy model [guinea pig (GP) model]. Top to bottom, action potential (AP), Ca^{2+} transient ($[Ca^{2+}]_i$), L-type calcium current ($I_{Ca,L}$) and the Na^+/Ca^{2+} exchanger current ($I_{Na/Ca}$).

the AP. In the RA model, the AP is so short that the Ca^{2+} -dependent inactivation contributes very little to the AP waveform. In both models, bigger Ca^{2+} transients modulate the current via $\text{Na}^+/\text{Ca}^{2+}$ exchange. In the RA model this current has a depolarizing effect at negative potentials (an inward current) and increases the AP duration. In the GP model the $I_{\text{Na}/\text{Ca}}$ is outward up to 100 ms after the onset of the AP. Thus NaCa exchange produces a repolarizing current shortening the AP in the GP model cell. This effect is influenced by the fact that I_{SAC} activation produces a Na^+ accumulation. At diastolic membrane potential the $[\text{Na}^+]_i$ increased from 10.9 to 11.5 mM in the RA model, and from 10.2 to 13.1 mM in the GP model. However, the change of the NaCa exchanger reversal potential on $[\text{Na}^+]_i$ increase is small compared with the increase of the Ca^{2+} transient.

Role of sodium accumulation

To simulate sodium accumulation by PKC-dependent Na^+/H^+ -exchanger activation we increased the diastolic $[\text{Na}^+]_i$ from 10.9 to 14 mM in the RA model and from 10.2 to 14 mM in the GP model, an intervention that produced an increase of the Ca^{2+} transients (Fig. 2). Similarly, as during SA channel activation in the GP model, APD_{90} is reduced from 143 to 114 ms because of the increased inactivation of the $I_{\text{Ca,L}}$ and increased outward $I_{\text{Na}/\text{Ca}}$, both caused by the augmented Ca transients. The duration of the AP in the RA model cell shows only minor changes on increased $[\text{Ca}^{2+}]_i$. The APD_{90} increases from 50 to 55 ms. This is contributed by the elevation of $[\text{Na}^+]_i$, which reduces inward $I_{\text{Na}/\text{Ca}}$ of the Ca^+ transient triggered by shifting the reversal potential of the exchanger to more positive potentials.

β -Adrenoceptor Stimulation

In the simulations of the β -receptor stimulation we used the two known effects via the PKA. First, the L-type Ca^{2+} channels are phosphorylated, promoting enhanced Ca^{2+} influx (increase of the L-type current). Second, the phosphorylation of the regulatory protein of the SERCA, the phospholamban, leads to increased SR Ca^{2+} uptake. These mechanisms result in a faster relaxation but also accumulate Ca^{2+} into the SR and thereby promote increased Ca^{2+} release during excitation.

β -Receptor stimulation was simulated by augmenting the $I_{\text{Ca,L}}$ and by increasing the SERCA activity (Fig. 3). Both model cells have similar responses to β -receptor stimulation, including Ca^{2+} accumulation in SR, which, in turn, leads elevated Ca^{2+} transients (2.12 μM in RA model and 2.10 μM in GP model) and prolongation of APD (APD_{90} increased to 64.0 ms from 50.1 ms in the RA model, and to 174 ms from 143 ms in the GP model). The mechanism of the AP lengthening is different in RA and GP models. In the RA model the AP lengthening was obviously caused by the $I_{\text{Na}/\text{Ca}}$ augmentation (Fig. 3A, bottom trace), whereas in the GP model the AP duration increased because of the reduced (here Ca^{2+} dependent) inactivation of the $I_{\text{Ca,L}}$ (Fig. 3B, third trace). The latter effect is the result of an increase in the maximal conductance of the L-type channels (to increase the current), when the Ca^{2+} transient-induced inactivation is countered and overcome by the increased voltage activation in the (now) longer APs.

Role of SR Ca^{2+} Content

The Ca^{2+} release and resulting Ca^{2+} transient seemed to exert a crucial influence on the AP shape in

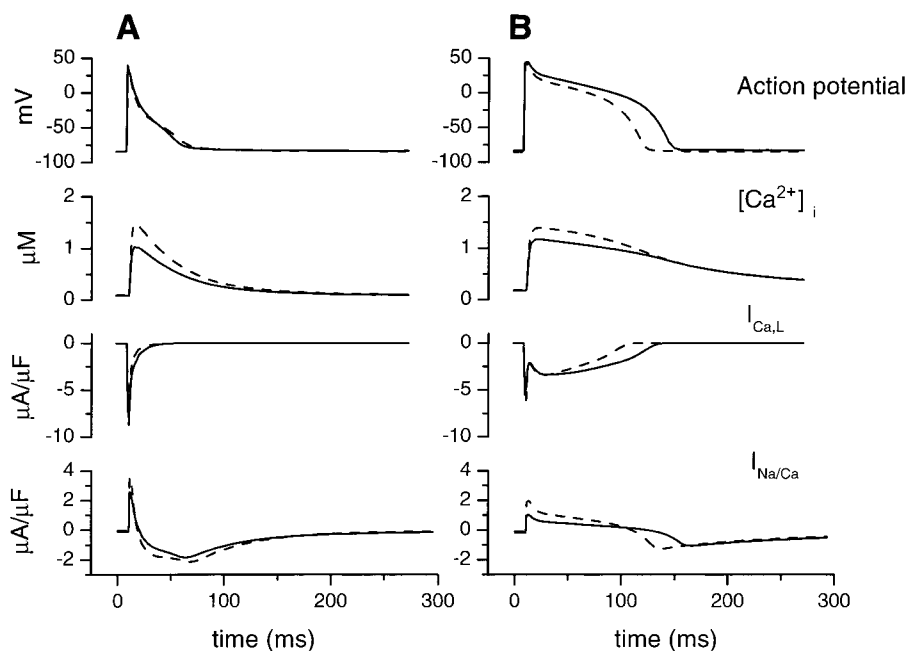


Fig. 2. Effect of increased intracellular Na^+ concentration ($[\text{Na}^+]_i$). Comparison of steady-state responses before (solid line) and after (dashed line) increasing $[\text{Na}^+]_i$ from 10 to 14 mM. A: RA model; B: GP model.

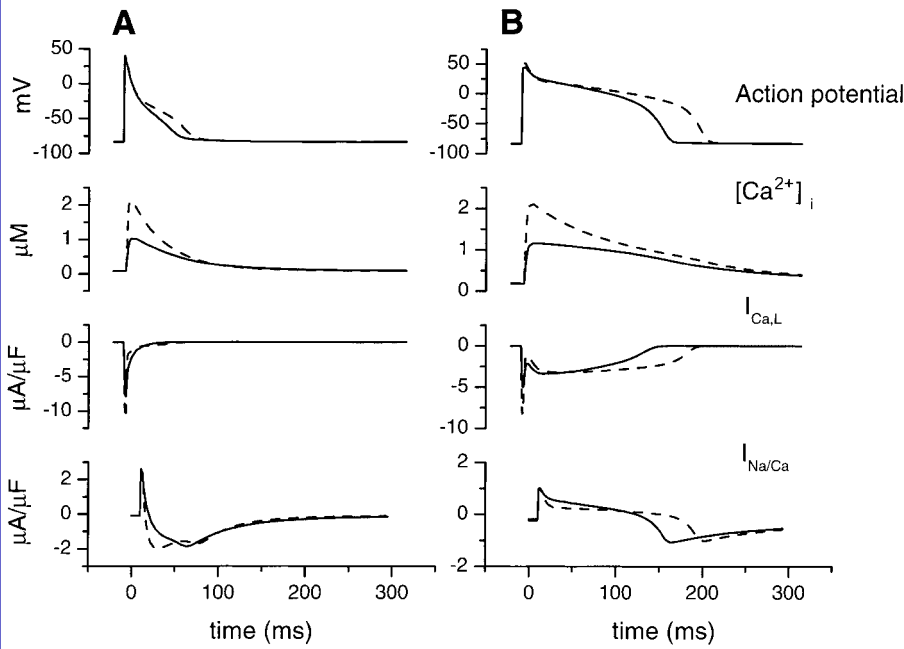


Fig. 3. Effects of β -adrenoceptor stimulation [both L-type Ca^{2+} conductance and the activity of the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) are increased]. Steady-state responses after simulation, control (solid line), simulated β -adrenoceptor stimulation (dashed line). A: RA model; B: GP model.

both models. Because the amount of Ca^{2+} released during excitation depends on the amount of the Ca^{2+} stored in SR (2, 3), we did the following simulation to evaluate the role of SR Ca^{2+} release in relation to all other studied mechanisms. To produce a Ca^{2+} transient increase the SR Ca^{2+} content was increased 20% in RA and 50% GP model cells (for scaling, see MATERIALS AND METHODS). At steady state, the peak of Ca^{2+} transient increased in both models, to $1.91 \mu\text{M}$ from $1.02 \mu\text{M}$ in the RA model and to $1.92 \mu\text{M}$ from $1.16 \mu\text{M}$ in the GP model (Fig. 4). In the RA model, the APD_{90} increased to 70.9 ms (27.9%) from 51.6 ms. In the GP model, APD_{90} shortened to 114 ms (20.9%) from 144 ms. In both models, AP duration changed

without significant changes in $[\text{Na}^+]_i$ or intracellular K^+ concentration ($[\text{K}^+]_i$). The ionic mechanism underlying the prolongation of APD in the RA model is that the peak of increased $[\text{Ca}]_i$ overlaps the late repolarization, generating an enhanced inward $I_{\text{Na}/\text{Ca}}$ together with a slight inactivation of $I_{\text{Ca},\text{L}}$. A greater $I_{\text{Na}/\text{Ca}}$ delays the repolarization at late phase of AP, resulting in the prolongation of late phase of AP. Meanwhile, in GP model cell, the increase of Ca^{2+} transient inactivates the $I_{\text{Ca},\text{L}}$ resulting the prominent shortening of the AP duration (Fig. 4). In the GP model, the enhanced inward $I_{\text{Na}/\text{Ca}}$ in this situation has only minor impact on the shape of the AP.

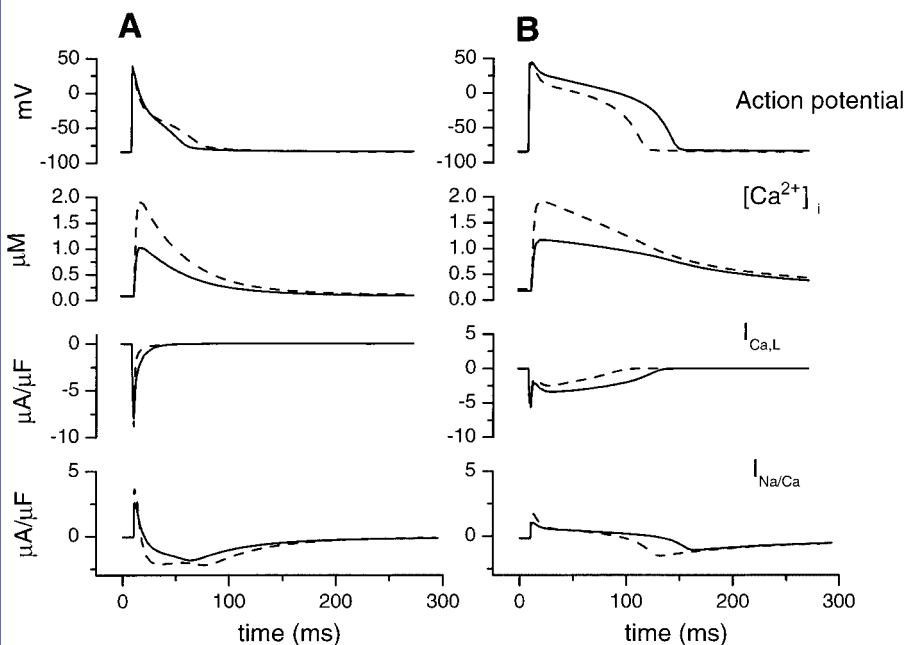


Fig. 4. Effects of increased sarcoplasmic reticulum (SR) Ca^{2+} content. Steady-state responses before (solid line) and after (dashed line) increasing Ca^{2+} in SR. A: RA model; B: GP model.

Clamping of $I_{Ca,L}$ and $I_{Na/Ca}$

On the basis of the simulation of the present study, it seemed that the Ca^{2+} transient modulates the transmembrane currents differently in the RA and GP models. To further study this, we tried to separate the contribution of $I_{Ca,L}$ and $I_{Na/Ca}$ on the duration of AP during an enlarged Ca^{2+} transient (Fig. 5, A–D). By increasing the SR Ca^{2+} load (similarly as in Fig. 4), the Ca^{2+} transient was increased from ~ 1 to $2 \mu M$ in both models (Fig. 5, B and D). As previously shown, the increase of the Ca^{2+} transient decreased the AP duration in the GP model (Fig. 5A) but increased duration in the RA model (Fig. 5C), in traces marked “SR $Ca \uparrow$.” The changes in APs were then analyzed further by clamping the enlarged Ca^{2+} transient at the same time as one of the key factors, either L-type current or the exchanger current was clamped to the same time course as the control situation. When the time course of $I_{Ca,L}$ was clamped, this caused a shift of the APD in the GP model toward the control value (Fig. 5A, top trace). When time course of $I_{Na/Ca}$ was clamped (Fig. 5A, bottom trace), the AP duration was shortened compared with control and with duration at higher Ca^{2+} transient. In identical simulations with the RA model (Fig. 5C), $I_{Na/Ca}$ clamp reduced the AP duration. Meanwhile, when $I_{Ca,L}$ was clamped, the APD was not significantly changed (traces marked as in Fig. 5A). The simulation suggests that in rat atrial cell, the prolongation of APD induced by elevated $[Ca^{2+}]_i$ transient is mainly caused by the change in the $I_{Na/Ca}$ rather than the sarcolemmal L-type current. In contrast, in the GP model clamping of $I_{Na/Ca}$ little affected the duration of the AP with elevated $[Ca^{2+}]_i$ transient, but the clamping of $I_{Ca,L}$ forced the APD back to control value. This implies that the reduction of APD in the GP model

induced by elevated $[Ca^{2+}]_i$ is mainly contributed by the increased inactivation of the $I_{Ca,L}$ rather than changes in the $I_{Na/Ca}$.

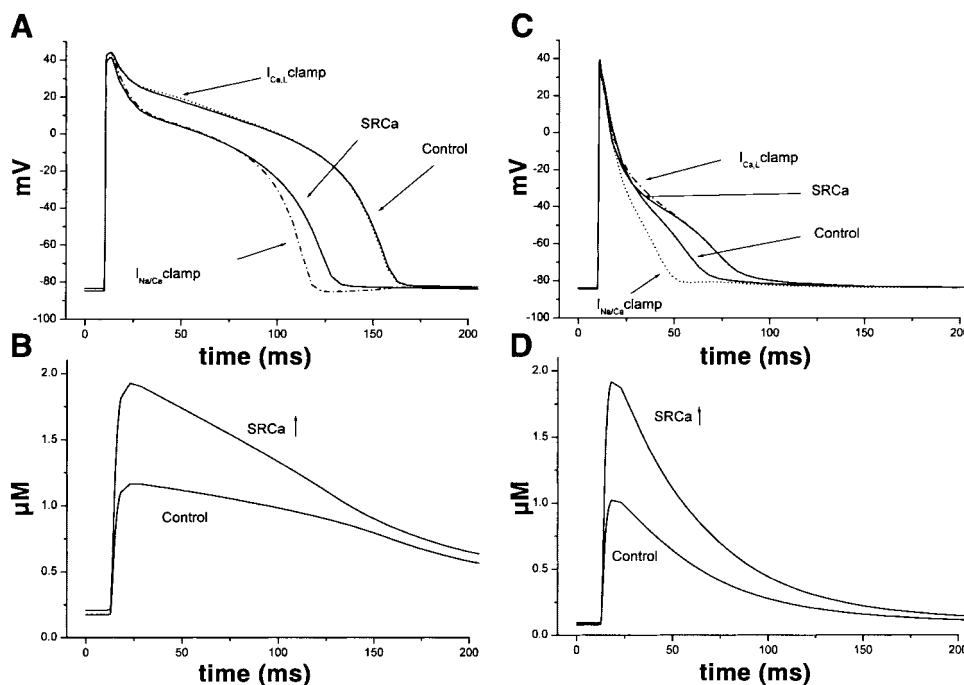
DISCUSSION

The present investigation demonstrates the importance of Ca^{2+} influx and accumulation in SR in modulating the length of AP of cardiac myocytes during inotropic interventions. In the modeled cells, activation of SA channels, increase of the $[Na^+]_i$, and SR Ca^{2+} accumulation all caused qualitatively similar changes in the shape of AP and augmentation of the Ca^{2+} transient. In the AP model, all of these interventions caused lengthening of the AP, whereas in GP model AP duration decreased. In contrast, simulated β -stimulation differs from all the other inotropic interventions because it produces AP lengthening in both models. We have built the simulations to keep the change in the Ca^{2+} transients about the same in all interventions, to facilitate comparison, and thus many effects, most probably that of β -receptor stimulation may be more prominent in real cells. On the basis of simulations, we can suggest that whatever is the mechanism promoting the changes in the Ca^{2+} transient the change in the AP shape is significantly contributed by the increase of the Ca^{2+} transient. The results also implicated that the AP duration is controlled differently in cardiac myocytes with short and long AP duration during inotropic interventions.

Species-Specific and Cell-Type Differences in Regulation of AP Waveform by $[Ca^{2+}]_i$

The durations of APs are generally much shorter in atria than in ventricles. In the rat atrium, the presence of a relatively large depolarization-activated outward

Fig. 5. Relationships between form of the AP, $[Ca^{2+}]_i$, $I_{Ca,L}$, and $I_{Na/Ca}$ during increase in Ca^{2+} stores. In this, APs (A and C) and Ca^{2+} transients (B and D) are shown for the GP model (A and B) and the RA model (C and D) with the control value (Control), and for when the Ca^{2+} transients were increased by increasing the Ca^{2+} in SR in the model as in Fig. 4 (SRCa \uparrow). The contribution of the clamped currents to the change in duration of the AP could be separated by clamping the $I_{Ca,L}$ and $I_{Na/Ca}$ to their time courses under the control conditions (dashed-dotted and dotted lines, respectively, in A and C). Note that $I_{Ca,L}$ clamp and Control traces are nearly identical in A, and same applies to the SRCa and Control traces in B.



current rapidly repolarizes the membrane potential (6), causing a lack of a prominent plateau phase. A long plateau is a characteristic of ventricular APs in most species, such as the guinea pig, rabbit, human, and dog (22). The inactivation of L-type Ca^{2+} channels is partly voltage dependent, and therefore, the rapid repolarization of (rat) atrial APs should cause $I_{\text{Ca,L}}$ to be inactivated faster than in the ventricles, as also observed by AP clamp in rat and rabbit (33). However, inactivation of $I_{\text{Ca,L}}$ depends not only on membrane voltage but also on $[\text{Ca}]_i$ (33, 39). Because of the faster repolarization of rat atrial AP, the L-type Ca^{2+} channel inactivation is mediated predominantly by voltage-dependent inactivation, with little or no contribution of the Ca^{2+} -dependent inactivation. Therefore, the inactivation of $I_{\text{Ca,L}}$ is much more sensitive to increased Ca^{2+} transients in the guinea pig ventricle than in the rat atrium, as shown in the present simulation studies. On the other hand, Ca^{2+} release accompanying APs of short duration as seen in rat atria, leads to a situation where the peak of Ca^{2+} transient overlaps the late repolarization, resulting in a peak inward $I_{\text{Na/Ca}}$ in late repolarization. Because of the plateau of the AP of the GP model, the Ca^{2+} transient triggers an $I_{\text{Na/Ca}}$ that is of outward direction during the entire duration of plateau. This leads to fundamental differences in the AP regulation by Ca^{2+} transient in the RA and GP models. As a manifestation of this, Ca^{2+} transient augmentation leads to decrease of the AP duration in the GP model, whereas the same stimuli in the RA model results in an increase of the AP duration.

Role of Different Ca^{2+} Sources in AP Modulation

As shown in the present study, AP shape depends, among other things, on the source of Ca^{2+} producing the increase of the Ca^{2+} transient. In the RA model, the increase of the AP duration results from the augmentation of the Ca^{2+} transient via inward $I_{\text{Na/Ca}}$ (Fig. 5). This effect shows no dependence on the source of Ca^{2+} producing the increase of the Ca^{2+} transient. However, in the GP model the AP shape depends strongly on the $I_{\text{Ca,L}}$. In the simulated β -stimulation (Fig. 3), where $I_{\text{Ca,L}}$ (and Ca^{2+} storage into SR) was increased (by increasing the maximal conductance), both models produced qualitatively similar changes in the AP, in line with the experimental findings from human atrial (18), guinea pig (21), and in rat ventricular cells (26). The mechanism of this was totally different in the RA model and the GP model. In the GP model, the AP lengthening was solely due to reduced inactivation of the L-type current caused by the increased voltage-dependent activation (Fig. 3). In the RA model, the AP is less dependent on the L-type current, and therefore, similar simulation strategy changes the AP by $I_{\text{Na/Ca}}$ due to the increased Ca^{2+} transient.

Possible Physiological Implications

AP shape of the cardiac myocyte reflects concerted activation and inactivation of many ion channels and

exchangers (3). In cardiac tissue, the length of APs also determines the excitability of the cells and the whole heart. Shortening or lengthening of AP can be proarrhythmic in the intact heart (32). The evident species-specific and atrial-ventricular-specific differences in the Ca^{2+} -dependent regulation of the AP duration may cause confusion, when different animal models of the heart are used. For example, it has been shown that stretch of cardiac tissue produces Ca^{2+} transient augmentation in variety of cardiac preparations (1, 13, 14, 27, 30). The effect of stretch on the cardiac myocyte AP is, however, dependent on the species (and to some extent, on its atrial or ventricular origin). As suggested by the present study, in species with short AP duration (mouse and rat) Ca^{2+} transient increase shortens the AP (especially in the atrium), whereas in species with long AP (guinea pig, rabbit, and human), similar increase in the Ca^{2+} transient is likely to increase the duration (again, especially in the ventricles). As shown previously, in rat atrial myocytes stretch lengthens the AP (27, 28) and in species with long AP, a reduction of the duration has been reported (11, 23, 29, 31, 34). Because stretch is considered to be proarrhythmic (10, 16, 17), it is likely that the mechanism of stretch-induced arrhythmias is different in different species. The similar dispersion of the AP in response to other inotropics is likely to exist between different species. These include action of hormones (ET-1, angiotensin II, and epinephrine) and transmitters (norepinephrine), and experimental manipulations that augment Ca^{2+} transients. In addition, the impact of ion channel modulators (blockers or activators) on the AP probably depends on the species, as suggested previously (12, 25). It is also notable that the murine transgenic models are used to, e.g., simulate the pathogenesis of human heart. Because there are fundamental differences in the excitation-contraction coupling of the rat and humans, generalizations of the mechanisms and successful application of the results obtained with transgenic techniques, require understanding of these differences.

In summary, the contribution of Ca^{2+} -dependent currents on the AP duration depends on the length of the AP. In short AP myocytes, Ca^{2+} transient augmentation promotes inward current via the $\text{Na}^+/\text{Ca}^{2+}$ exchange, which lengthens the AP. This effect is similar regardless of the mechanism causing the Ca^{2+} transient increase. In myocytes with long APs the increase of Ca^{2+} transients as such decreases the AP duration via Ca^{2+} -dependent inactivation of the $I_{\text{Ca,L}}$. However, large increase in the L-type current increases the AP duration (as with β -stimulation) despite the simultaneous Ca^{2+} transient augmentation.

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