A Dynamic Model of the Cardiac Ventricular Action Potential

II. Afterdepolarizations, Triggered Activity, and Potentiation

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Abstract The action potential model presented in our accompanying article in this journal is used to investigate phenomena that involve dynamic changes of [Ca2+], as described below. Delayed afterdepolarizations (DADs) are induced by spontaneous Ca2+ release from the sarcoplasmic reticulum (SR), which, in turn, activates both the Na+-Ca2+ exchanger (INaCa) and a nonspecific Ca2+-activated current (I_{NaCa}). The relative contributions of INaCa and I_{NaCa} to the generation of DADs are different under different degrees of Ca2+ overload. Early afterdepolarizations (EADs) can be categorized into two types: (1) plateau EADs, resulting from a secondary activation of the L-type Ca2+ current during the plateau of an action potential, and (2) phase-3 EADs, resulting from activation of I_{NaCa} and I_{NaCa} by increased [Ca2+], due to spontaneous Ca2+ release from the SR during the late repolarization phase. Spontaneous rhythmic activity and triggered activity are caused by spontaneous Ca2+ release from the SR under conditions of Ca2+ overload. Postextrasystolic potentiation reflects the time delay associated with translocation of Ca2+ from network SR to junctional SR. The cell is paced at high frequencies to investigate the long-term effects on the intracellular ionic concentrations. (Circ Res. 1994; 74:1097-1113.)

Key Words • afterdepolarizations • postextrasystolic potentiation • triggered activity

The model of the mammalian ventricular action potential that was introduced in our accompanying article in this journal (the L-R model) provides an accurate description of dynamic changes in ionic concentrations and ionic fluxes. In particular, processes that regulate the dynamics of intracellular Ca2+ transients during the action potential are simulated correctly by the model. In addition, the model accounts for the effects of intracellular Ca2+ changes on ionic currents (eg, Ca2+-dependent inactivation of Ca2+ current through the sarcolemma, Na+-Ca2+ exchange current [INaCa], and nonspecific Ca2+-activated current [I_{NaCa}]). These properties of the model make it possible to study, using simulations, physiological phenomena that are related to the excitation-contraction coupling process in cardiac myocytes. It is also possible to simulate the behavior of the cell under conditions of Ca2+ overload and to suggest mechanisms of related arrhythmogenic activity of the single cell (eg, early afterdepolarizations [EADs], delayed afterdepolarizations [DADs], and triggered activity).

The effects of the stimulation pattern on the contractile strength of extrasystoles and postextrasystoles were studied in tissue preparations since the 1960s. As the extrasystole becomes more premature, the strength of muscle contraction at the postextrasystole is increased if the interval between the extrasystole and the postextrasystole is fixed. This phenomenon, known as postextrasystolic potentiation, was demonstrated recently in tissue preparations and in single cells and was explained by an excitation-contraction coupling model. However, the underlying mechanism of this phenomenon was not elucidated clearly, and the dynamic processes that control this behavior were not characterized in a quantitative way. In the present article, we use the L-R model to simulate this behavior to explain the underlying mechanism in terms of dynamic processes such as Ca2+ release and uptake by the sarcoplasmic reticulum (SR), translocation of Ca2+ between compartments in the SR, and buffering processes in the SR and the myoplasm.

DADs and EADs are single-cell phenomena that can induce abnormal rhythmic activity and cardiac arrhythmias. An afterdepolarization is defined as a depolarizing afterpotential that occurs before (early) or after (delayed) the completion of action potential repolarization. Also, rhythmic activity, known as triggered activity, can be induced by pacing a quiescent cell for a certain time period. All of these arrhythmogenic phenomena involve dynamic changes in intracellular Ca2+, such as spontaneous Ca2+ release from the SR and activation of membrane currents through a Ca2+-activated process. These processes are incorporated in our model, and their complex effects (these processes interact and influence each other, in addition to their direct effect on the cell membrane) on the action potential can be simulated. In the present article, we use the L-R model to characterize and investigate the mechanisms underlying arrhythmogenic behavior of the single myocyte under a variety of conditions and different degrees of Ca2+ overload.

Materials and Methods

Methods for computing the action potential and the formulation of underlying processes were described in detail in our accompanying article. However, properties and formulation
of processes that play an important role in the phenomena studied in the present article are described briefly below.

The model consists of ionic currents through the sarcolemma and of Ca$^{2+}$ fluxes inside the cell. Sarcolemmal currents of importance to phenomena simulated here are $I_{\text{Ca}}$, $I_{\text{NaCa}}$, and $I_{\text{NaCa}}$ (explained more fully below). Intracellular fluxes of Ca$^{2+}$ ions are the basis for many of the phenomena investigated in the present article and, through interactions with the sarcolemmal currents, affect the action potential. Equations formulating the most relevant currents and Ca$^{2+}$ fluxes in the cell are listed in Appendix 1. Symbols used in the equations and throughout this article are defined in Appendix 2.

$I_{\text{Ca}}$, Ca$^{2+}$ Current Through the L-type Channel

Based on recent experimental data, activation of $I_{\text{Ca}}$ in our model is very fast. It can be activated in 2 milliseconds, an order of magnitude faster than in the Beeler and Reuter action potential model. Inactivation is formulated to be dependent on the membrane potential through a voltage-dependent gate (f-gate) and on [Ca$^{2+}$], through a Ca$^{2+}$-dependent gate (f$_{\text{Ca}}$-gate). Therefore, the recovery of $I_{\text{Ca}}$ from inactivation depends not only on membrane potential but also on the level of [Ca$^{2+}$].

$I_{\text{NaCa}}$ and $I_{\text{NaCa}}$, Ca$^{2+}$-Activated Currents Through the Sarcolemma

In our model, two sarcolemmal currents are activated by an increase of [Ca$^{2+}$], These are $I_{\text{NaCa}}$ (the Na$^{+}$-Ca$^{2+}$ exchange current) and $I_{\text{NaCa}}$ (the nonspecific Ca$^{2+}$-activated current). The total [Ca$^{2+}$]-activated inward current, known as IA, is defined as the sum of these two currents: $I_A=I_{\text{NaCa}}+I_{\text{NaCa}}$.

Ca$^{2+}$ Release From the Junctional SR

Ca$^{2+}$ release ($I_{\text{JSR}}$) from the junctional SR (JSR) can be triggered in two different ways, externally and internally. An external triggering process is a sufficiently fast increase of the myoplasmic [Ca$^{2+}$], known as the Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) process. The CICR process is initiated by Ca$^{2+}$ entry through the sarcolemmal channels. An internal triggering process involves Ca$^{2+}$ overloading of the JSR above a threshold level, a process known as spontaneous Ca$^{2+}$ release. Because of their different triggering mechanisms, the formulations of these release processes are somewhat different. In Appendix 1, both share the same form: $I_{\text{JSR}}=g_{\text{JSR}} \cdot ([Ca^{2+}]_{JSR}-[Ca^{2+}]_{\text{JSR}}) \cdot \text{mmol/L per millisecond}$. However, their rate constants $(g_{\text{JSR}})$ are different. The major differences are the values of the maximum rate constants $(g_{\text{JSR}})$ and the Hill equation used to describe the CICR process. Parameter values are adjusted to obtain a peak intracellular Ca$^{2+}$ transient of 1 μmol/L under normal conditions.

Ca$^{2+}$ Fluxes During an Action Potential

In the model, the SR is divided into two compartments: network SR (NSR) and JSR. Once the cell is excited, Ca$^{2+}$ ions enter the cell through the $I_{\text{Ca}}$, bringing about a small increase of [Ca$^{2+}$]. If the increase 2 milliseconds after $V_{\text{mem}}$ (indicated by $\Delta$[Ca$^{2+}$]) reaches a threshold level ($\Delta$[Ca$^{2+}]_t=0.18 \mu\text{mol/L}$), Ca$^{2+}$ release from the JSR is triggered by the CICR process. This causes a transient ([Ca$^{2+}$]$_{t}=1 \mu\text{mol/L}$) under normal conditions. The released Ca$^{2+}$ then reenters the JSR through an uptake mechanism. It is then transferred to the JSR through a translation process with a time constant of 180 milliseconds. If JSR is Ca$^{2+}$-loaded to a threshold level, spontaneous Ca$^{2+}$ release from the JSR occurs. Otherwise, calcium is transferred back from the JSR to the NSR and leaks to the myoplasm through a leakage channel ($I_{\text{Ca}}$). Ca$^{2+}$ ions that remain in the myoplasm are extruded from the cell by the exchange $I_{\text{NaCa}}$ after completion of the action potential repolarization phase. [Ca$^{2+}$]$_{t}$ returns to a low resting level of 0.12 μmol/L.

Ca$^{2+}$ Buffers in the SR and in the Myoplasm

The model includes buffering of Ca$^{2+}$ ions both in the myoplasm and in the SR. Ca$^{2+}$ buffers in the myoplasm (calmodulin and troponin) regulate [Ca$^{2+}$]$_{t}$ to be maintained at an appropriate level (a peak transient of 1 μmol/L under normal conditions). Ca$^{2+}$ buffer in the SR (calcineurin (CSQN)) increases the Ca$^{2+}$ storage capacity of the JSR, so that the amount of Ca$^{2+}$ released is sufficient to cause muscle contraction. Also, the JSR buffer allows Ca$^{2+}$ ions to be loaded efficiently into the SR when the cell is paced at fast frequencies. Equations governing the buffering processes are provided in Appendix 1.

$I_{\text{Ca}}$, Total Time-Independent Current Through the Sarcolemma

$I_{\text{Ca}}$ consists of six ionic currents, $I_{\text{Ks}}$, $I_{\text{Kp}}$, $I_{\text{NaK}}$, $I_{\text{NaCa}}$, $I_{\text{NaCa}}$, and $I_{\text{Ca}}$ (see Appendix 2 for definitions). The major components of $I_{\text{Ca}}$ are $I_{\text{Ks}}$ at negative potentials and $I_{\text{Kp}}$ at plateau potentials. $I_{\text{Ca}}$ develops a phase of negative slope of its current-voltage curve that is a property of its $I_{\text{Ks}}$ component. The conductance of $I_{\text{Ks}}$ depends on the $[K+]_p$.

Results

Postextrasystolic Mechanical Potentiation

The phenomenon of postextrasystolic mechanical potentiation was used to study certain aspects of the excitation-contraction coupling process in cardiac muscle. The following is a model simulation of the postextrasystolic potentiation phenomenon. By pacing the cell at a basic cycle length (BCL) of 500 milliseconds, the cell is primed (loaded) with Ca$^{2+}$ ions that accumulate in the SR. The pacing period required for the cell to reach a dynamic steady state in the simulations is at least 10 seconds. After the pacing period, two additional beats are introduced: an extrasystole at a coupling interval ($T_{\text{esi}}$) from the onset of stimulation of the last paced beat and a postextrasystole at a coupling interval ($T_{\text{pi}}$) from the onset of stimulation of the extrasystolic beat (the protocol is described in Figs 1A and 2A). In Fig 1, for a fixed $T_{\text{esi}}$, postextrasystolic Ca$^{2+}$ release from the JSR increases exponentially to a plateau level (Fig 1B) as $T_{\text{pi}}$ is increased from 220 to 2000 milliseconds. This phenomenon is known as postextrasystolic restitution of Ca$^{2+}$ release. The underlying mechanism of the monoexponential restitution is studied in Fig 2. The priming period in Fig 2 lasts for only four beats, resulting in an increase of [Ca$^{2+}$] in the NSR from the resting value of 1.73 to 2.094 mmol/L (empty arrow in panel B). For a fixed interval ($T_{\text{esi}}=500$ milliseconds), [Ca$^{2+}$]$_{\text{NSR}}$ is increased to a peak value of 2.13 mmol/L after the extrasystole. Ca$^{2+}$ then translocates from the NSR to the JSR according to a monoexponential time course with a time constant of 180 milliseconds. This gradual translocation of Ca$^{2+}$ ions from the NSR to the JSR explains the monoexponential restitution of Ca$^{2+}$ release from the JSR observed in Fig 1 as $T_{\text{pi}}$ is increased. Note that at the onset of the extrasystole (marked by an asterisk in Fig 2), NSR has lost a portion of its Ca$^{2+}$ to the JSR so that [Ca$^{2+}$]$_{\text{NSR}}$ is decreased from 2.094 mmol/L (empty arrow, panel B) to 2.022 mmol/L (asterisk in panel B). As $T_{\text{esi}}$ is decreased and the extrasystole is applied earlier, total Ca$^{2+}$ translocation from the NSR to the JSR during $T_{\text{esi}}$ is decreased as well, and a larger portion of the NSR Ca$^{2+}$ content translocates into the JSR during $T_{\text{pi}}$, resulting in an increased JSR release during the postextrasystole.


Fig 1. Tracings showing postextrasystolic restitution of Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR). A shows the membrane potential (V); B, [Ca\(^{2+}\)]\(_{i}\), in the myoplasm. The priming period is 10 seconds for the cell to reach a dynamic steady state. To construct the postextrasystolic restitution curve, the extrasystolic coupling interval (T\(_{sw}\)) is fixed (T\(_{sw}=250\) milliseconds in the figure), and the postextrasystolic coupling interval (T\(_{pes}\)) is varied from 220 to 2000 milliseconds. Δ[Ca\(^{2+}\)]\(_{i}\), defined as the peak intracellular Ca\(^{2+}\) transient of the postextrasystolic action potential minus [Ca\(^{2+}\)]\(_{i}\) just before the postextrasystole, provides a measure of Ca\(^{2+}\) release from the junctional SR at each postextrasystole. The monotonic increase of Δ[Ca\(^{2+}\)]\(_{i}\) with T\(_{pes}\) is the restitution curve. T\(_{sw}\) and T\(_{pes}\) are measured between onsets of the respective stimuli and are defined in panel A (see also Fig 2A).

explains the mechanism of postextrasystolic potentiation observed in cardiac muscle.\(^{3,4}\) The results for different values of T\(_{sw}\) are summarized in Fig 3. Clearly, as T\(_{sw}\) decreases from 1500 to 250 milliseconds, the monoeponential restitution curve shifts upward, indicating that more Ca\(^{2+}\) ions are released from the JSR during the postextrasystole. The time constant of these curves is 253±18 milliseconds (mean±SD). Also, T\(_{pes}\) is reduced, note the leftward shift of the restitution curves (denoted by an arrow). This shift is caused by the shortening of the extrasystolic action potential duration (APD) from 170.5 to 135 milliseconds, which is due to its increased prematurity.\(^{10}\) Therefore, as T\(_{sw}\) decreases, the minimum value of T\(_{pes}\) required to obtain a successful Ca\(^{2+}\) release after the extrasystole is decreased, resulting in a leftward shift of the curve. This shift is consistent with the experimental findings by Yue et al.\(^{3}\)

**Spontaneous Ca\(^{2+}\) Release From the JSR and Its Relation to EADs and DADs**

**Normal Conditions (No Ca\(^{2+}\) Overload)**

As discussed by Stern et al.,\(^{11}\) the phenomenon of spontaneous Ca\(^{2+}\) release from the JSR is universal in mammalian myocardium, including atria, Purkinje fibers, and ventricles and also in skinned cardiac preparations.\(^{12}\) The characteristics of spontaneous Ca\(^{2+}\) release are most easily observed in isolated cardiac myocytes from the rat under normal conditions (normal [Ca\(^{2+}\)]). In our model, most of the free Ca\(^{2+}\) ions in the JSR are buffered by CSON so that 68.5% of [CSON] is Ca\(^{2+}\) bound in the resting state. By setting the threshold for spontaneous release ([CSON]\(_{th}\)) at 70%, we readily observe spontaneous Ca\(^{2+}\) release from the JSR as a result of pacing the cell under normal conditions. The same behavior was observed experimentally in the rat.\(^{11,13-15}\) As shown in Fig 2, NSR can be loaded with Ca\(^{2+}\) ions as a result of pacing. If the degree of Ca\(^{2+}\) loading is such that [CSON] that is Ca\(^{2+}\) bound is greater than [CSON]\(_{th}\), spontaneous release occurs (note that Ca\(^{2+}\) loading of NSR implies Ca\(^{2+}\) loading of the JSR through the translocation process between these two compartments). In Fig 4, the cell is paced by a train of stimuli at a BCL of 400 milliseconds for 11 beats, and then the pacing is terminated. As a result, DADs are observed with coupling intervals of 1040 and 1085 milliseconds and with amplitudes of 2.6 and 2.3
This DAD threshold depends on the percentage buffering of CSQN at the JSR beyond which release occurs ([CSQN]_{NSR}). A higher [CSQN]_{NSR} requires that more Ca^{2+} ions translocate from the NSR to the JSR before [CSQN]_{NSR} is reached, implying a higher DAD threshold of the [Ca^{2+}]_{NSR}. Under normal conditions, the peak intracellular Ca^{2+} transient caused by one spontaneous Ca^{2+} release is only 0.54 μmol/L, and I_{NaCa} is only partially activated (~1/12 of its fully activated value). Therefore, its amplitude is only ~0.49 μA/μF, less than one half the I_{NaCa} amplitude of ~1.13 μA/μF. This means that I_{NaCa} is more important than I_{CaCa} in generating the DAD under normal conditions. Note that during the pacing period, the peak intracellular Ca^{2+} transient (panel B) increases monotonically from 0.68 μmol/L (second beat) to 0.8 μmol/L (last driven beat). This reflects the loading of NSR (panel E) that (through the translocation process) provides more Ca^{2+} ions for release from the JSR.

**Minimum Ca^{2+}-Overload Conditions**

I_{CaCa} is detected under Ca^{2+}-overload conditions, defined as resting [Ca^{2+}]_{o}≥0.3 μmol/L. In this section, we investigate the cellular responses under minimal Ca^{2+} overload by setting [Ca^{2+}]_{o}=0.3 μmol/L at the resting state. For this condition, [Ca^{2+}]_{NSR}=3.69 mmol/L and 80% of total [CSQN] in JSR binds Ca^{2+}. This situation can be achieved experimentally by increasing [Ca^{2+}]_{o}, to load the cell. As a result of the overload, the first action potential shown in Fig 5A is generated by spontaneous Ca^{2+} release and is followed by an EAD and a DAD before a stimulus (indicated by a vertical bar at the bottom of panel A) is applied. Peak I_{CaCa} associated with the initiation of the spontaneous action potential is only 0.6 μA larger than peak I_{NaCa} (panel E). Therefore, under minimum overload conditions, both currents are equally important for the generation of a spontaneous action potential, although I_{NaCa} is somewhat larger. The EAD at the plateau phase of the spontaneous action potential (takeoff at ~21.6 mV with amplitude of 19.4 mV) is generated by the double-peak behavior of I_{Ca} (asterisk in panel C) in contrast to the single-peak (normal) behavior of I_{Ca} (pound sign in panel C) for the action potential elicited by the stimulus. This comparison clearly demonstrates that secondary activation of I_{Ca} during the action potential generates the plateau EAD. This secondary activation results, in part, from the fast decrease of the intracellular Ca^{2+} transient (asterisk in panel B) that is due to uptake by the NSR (note that I_{NaCa} (panel E) is almost zero at the plateau phase and does not contribute to the decrease of [Ca^{2+}]_{o}). The decrease of [Ca^{2+}]_{o} causes recovery of I_{Ca} through the Ca^{2+}-dependent f-gate. At the same time, the f-gate also recovers, following its voltage-dependent characteristics. The underlying mechanism of this type of plateau EAD will be elucidated further below. Note that peak I_{Ca} of the paced beat is larger than that of the first (spontaneous) beat. The larger I_{Ca} during the paced beat results from the lower [Ca^{2+}]_{o}, which, in turn, results in a smaller degree of inactivation of I_{Ca} through the Ca^{2+}-dependent inactivation gate (f_{Ca}-gate).

By applying a stimulus at the decaying phase of the first DAD (Fig 5A), an action potential is elicited. After this action potential, a DAD of a relatively large ampli-

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**Fig 4.** Tracings showing delayed afterdepolarizations (DADs) after pacing under normal conditions (threshold for spontaneous release of calsequestrin, 70%; pacing, basic cycle length of 400 milliseconds). Spontaneous sarcoplasmic reticulum (SR) Ca^{2+} release occurs twice (arrows in panel B) to generate two DADs (arrows in panel A). The amplitude of the Na^{+}-Ca^{2+} exchange current (I_{NaCa}) during DADs is more than twice the amplitude of the nonspecific Ca^{2+}-activated current (I_{CaCa}). DAD threshold (empty arrow in panel E) is defined as the value of [Ca^{2+}]_{NSR}, where NSR is network SR, that generates a single DAD. A shows membrane potential (V); B, [Ca^{2+}]; C, I_{NaCa}; D, I_{CaCa}; E, [Ca^{2+}]_{NSR}; and F, Ca^{2+} content of the junctional SR ([Ca^{2+}]_{JSR}) (100% implies 10 mmol/L). Currents shown in the figures are for 1 μF of membrane capacitance.

The minimum [Ca^{2+}]_{NSR} that can cause spontaneous Ca^{2+} release from the JSR to be 2.13 mmol/L (empty arrow, panel E), which is the peak value in the interval between the last two spontaneous release episodes. This threshold value of [Ca^{2+}]_{NSR} can cause only one spontaneous Ca^{2+} release, resulting in one (the last) DAD. We refer to this value as the DAD threshold of [Ca^{2+}]_{NSR}. mV, respectively. The coupling interval is defined as the interval from the upstroke of a driven action potential to the peak of a DAD or to the upstroke of a resulting nondriven action potential. Clearly, these two DADs are associated with spontaneous Ca^{2+} release (panel B) from the overloaded JSR (panel F). As shown in panel E, NSR is loaded with Ca^{2+} ions to 2.42 mmol/L at the last paced beat (full arrow). Then, translocation of Ca^{2+} from the NSR loads the JSR (panel F). When JSR is loaded up to the 70% threshold of buffered CSQN, spontaneous Ca^{2+} release occurs, bringing about an increase of [Ca^{2+}]_{o} (panel B). This increase in [Ca^{2+}]_{o} activates both I_{NaCa} (panel C) and I_{CaCa} (panel D). These two depolarizing currents result in the DADs shown in panel A (arrows). Note that [Ca^{2+}]_{NSR} decreases monotonically toward its resting level (panel E) as a result of Ca^{2+} extrusion by I_{NaCa}. After two episodes of spontaneous Ca^{2+} release, [Ca^{2+}]_{NSR} is reduced almost to its resting value of 1.73 mmol/L. As a result, JSR cannot be overloaded to the threshold level, and additional episodes of spontaneous release cannot occur. We compute the minimum [Ca^{2+}]_{NSR} that can cause spontaneous Ca^{2+} release from the JSR to be 2.13 mmol/L (empty arrow, panel E), which is the peak value in the interval between the last two spontaneous release episodes. This threshold value of [Ca^{2+}]_{NSR} can cause only one spontaneous Ca^{2+} release, resulting in one (the last) DAD. We refer to this value as the DAD threshold of [Ca^{2+}]_{NSR}.
Fig 5. Tracings showing cellular responses under minimum Ca$^{2+}$ overload (both Na$^{+}$-Ca$^{2+}$ exchange current $I_{\text{NaCa}}$ and nonspecific Ca$^{2+}$-activated current $I_{\text{INaCa}}$ included). An action potential is generated by spontaneous Ca$^{2+}$ release, followed by an early afterdepolarization (EAD, empty arrow in panel A) and a delayed afterdepolarization (DAD, first full arrow, panel A) before a stimulus is applied. The EAD is generated by the double-peak behavior of the L-type Ca$^{2+}$ current ($I_{\text{CaL}}$, asterisk in panel C) that is very different from the single-peak $I_{\text{CaL}}$ (pound sign in panel C) of the driven action potential. The stimulus excites an action potential but fails to trigger Ca$^{2+}$ release from the junctional sarcoplasmic reticulum through the Ca$^{2+}$-induced Ca$^{2+}$ release process (pound sign in panel B). In contrast, Ca$^{2+}$ release occurs twice during the spontaneous action potential (asterisk in panel B). The stimulus is indicated by the vertical bar at the bottom of panel A. A shows membrane potential (V); B, [Ca$^{2+}$], in the myoplasm; C, $I_{\text{INaCa}}$, D, $I_{\text{INaCa}}$; and E, $I_{\text{INaCa}}$. Currents shown in the figures are for 1 $\mu$F of membrane capacitance.

titude is generated, followed by additional four DADs of monotonically decreasing amplitudes (Fig 5A). This decrease in amplitude of the subsequent DADs was also observed experimentally by Stern et al.$^{11}$ Note that in contrast to the plateau EAD, the DADs do not involve $I_{\text{CaL}}$ at all (panel C) but result from the depolarizing currents $I_{\text{INaCa}}$ and $I_{\text{INaCa}}$ (panels D and E, respectively).

To elucidate the importance of $I_{\text{INaCa}}$ in generating the spontaneous action potential, we set $I_{\text{INaCa}}$ = 0 in the model and ran the same simulation as in Fig 5, for the same minimum-overload conditions. The results are shown in Fig 6. Without $I_{\text{INaCa}}$, spontaneous Ca$^{2+}$ release fails to generate an action potential, and a sequence of spontaneous small depolarizations is observed (panel A). Note that the Ca$^{2+}$ content of the NSR (panel D) decreases monotonically because the steady state of the cell is the normal resting state with [Ca$^{2+}$] = 0.12 $\mu$mol/L and [Ca$^{2+}$]$_{\text{NSR}}$ = 1.73 mmol/L. Therefore, the overload state will gradually change to the normal resting state as a result of Ca$^{2+}$ extrusion by $I_{\text{INaCa}}$. In Fig 7, $I_{\text{INaCa}}$ was set to zero, but the amplitude of $I_{\text{INaCa}}$ was raised to $-62$ $\mu$A/µF (by increasing $k_{\text{INaCa}}$ [see Appendix 2] 2.5-fold). This $I_{\text{INaCa}}$ is equal to the sum of $I_{\text{INaCa}}$ and $I_{\text{INaCa}}$ in the simulation of Fig 5. As a result, an action potential is elicited by the enlarged $I_{\text{INaCa}}$, with no contribution from $I_{\text{INaCa}}$ (Fig 7A). This spontaneous action potential is followed by an EAD at the late repolarization phase (phase-3 EAD), which is very different from the EAD that appeared during the plateau phase in Fig 5. The takeoff potential of this EAD is $-64$ mV, located in the late repolarization phase where $I_{\text{Ca}}$ has been inactivated (in contrast to the significant $I_{\text{Ca}}$ at the onset of the plateau EAD, Fig 5C). The mechanism of late EADS is the same as that of DADs; both are generated by spontaneous Ca$^{2+}$ release from the JSR. The difference between them is that DADs are generated at the postrepolarization phase, whereas phase-3 EADs appear at the late repolarization phase where $I_{\text{v}}$ exhibits a negative slope. Note that the shape of the phase-3 EAD (Fig 7A) mirrors the (inverted) shape of $I_{\text{v}}$ in panel E. To investigate the role of the negative slope of $I_{\text{v}}$ in generating the phase-3 EADs, we eliminated (Fig 8) the negative slope of $I_{\text{v}}$ (and, therefore, of $I_{\text{v}}$) at the phase where the phase-3 EAD was elicited (see asterisk in Fig 8D and 8E). As shown in Fig 8A, the amplitude (8 mV) and the duration (41 milliseconds) of the phase-3 EAD are greatly reduced compared with those (24.4 mV and 110 milliseconds) supported by the negative slope of $I_{\text{v}}$ in Fig 7A. We conclude, therefore, that the negative slope of $I_{\text{v}}$ contributes in a major way to the amplitude and duration of phase-3 EADs. In contrast, DADs are generated at the postrepolarization phase, where the (almost constant)
**High Level of Ca\(^{2+}\) Overload**

In the case of high-level Ca\(^{2+}\)-overload conditions (resting [Ca\(^{2+}\)] is set at 0.4 \(\mu\)mol/L in the model), we observe spontaneous rhythmic activity resulting from spontaneous Ca\(^{2+}\) release from the JSR (Fig 9). Except for the first action potential that is elicited from the resting state, all other action potentials display a very short duration (short APD), and the BCL of the rhythmic activity increases monotonically until failure occurs and a DAD is observed. The short duration of these action potentials is caused by the high value of the activation X-gate of the time-dependent K\(^+\) current, \(I_K\), at the time of their onset (Fig 9D), a phenomenon known as postpolarization refractoriness. A short APD implies small total Ca\(^{2+}\) entry into the cell during the action potential. As a result of the increased imbalance between Ca\(^{2+}\) extrusion by \(I_{Ca}\) and the reduced Ca\(^{2+}\) entry, [Ca\(^{2+}\)]\(_{JSR}\) decreases monotonically (except for the long-duration first action potential) until the rhythmic activity ceases and a DAD is observed. Therefore, we can define a threshold for NSR overload for which an action potential can be elicited by spontaneous Ca\(^{2+}\) release from the SR. The peak intracellular Ca\(^{2+}\) transient at the last action potential is 1.28 \(\mu\)mol/L; it is
EADs is clearly showed (panel C). A shows membrane potential (V); B, [Ca$^{2+}$], in the myoplasm; C, $I_{Ca}$; D, $I_{k}$, activation gate (X); and E, concentration of Ca$^{2+}$ ions in the network sarcoplasmic reticulum ([Ca$^{2+}$]$_{NSR}$). Currents shown in the figures are for 1 μF of membrane capacitance.

1.26 μmol/L for the DAD (these values are indicated in Fig 9B). This small difference (0.02 μmol/L) results in a success or a failure to elicit an action potential. The action potential threshold for Ca$^{2+}$ in NSR can be determined as the value for which the last rhythmic action potential occurs. This value is 3.84 mmol/L (arrow in panel E). This threshold does not depend on the value of [Ca$^{2+}$]$_{SA}$ that determines Ca$^{2+}$ release from the overloaded JSR, since [Ca$^{2+}$]$_{SA}$ is reached even for Ca$^{2+}$ in NSR < action potential threshold, resulting in a DAD (Fig 9). In this respect, the action potential threshold is very different from the DAD threshold defined for normal (no Ca$^{2+}$ overload) conditions (see "Normal Conditions"). It only depends on the characteristics of the Ca$^{2+}$-activated currents ($I_{Ca}$ and $I_{NaCa}$) that are activated by the intracellular Ca$^{2+}$ transient to elicit an action potential.

After the cessation of the rhythmic activity, we pace the cell at a BCL of 600 milliseconds (Fig 10). In Fig 10A, plateau EADs (empty arrows) appear in a 2:1 pattern. Similar patterns were observed experimentally. These EADs disappear when the Ca$^{2+}$-overload condition is no longer maintained (panels A and E). It is clearly demonstrated that the appearance of plateau EADs is related to recovery of $I_{Ca}$ that results in a secondary peak inward current during the plateau of long-duration action potentials (empty arrows in panel C). The recovery of $I_{Ca}$ during the plateau phase (asterisk in panel C) is made possible by a significantly fast decrease of the intracellular Ca$^{2+}$ transient (asterisk in panel B), which results in recovery of the Ca$^{2+}$-dependent inactivation gate, $g_{Ca}$. This relatively fast recovery of inward $I_{Ca}$ on a background of a slow and small increase of the X-gate (asterisk in panel D) and, as a result, of the outward $I_{K}$ (not shown) results in a net inward (depolarizing) current that elicits the EAD. In Fig 10, the peak intracellular Ca$^{2+}$ transient and the rate of decrease of the intracellular Ca$^{2+}$ transient from its peak value are 1.7 μmol/L (indicated in panel B) and 6 μmol/L per second, respectively, for the first action potential that displays an EAD. The values are 1.65 μmol/L and 5.83 μmol/L per second, respectively, for the third beat with an EAD. The peak intracellular Ca$^{2+}$ transients and [Ca$^{2+}$], decrease rates of other beats that do not display an EAD (pound sign in panel B) are much smaller because of the short interval between the stimulus and the previous DAD (panel A). The JSR Ca$^{2+}$ content is depleted by the spontaneous release during the DAD. Because of the short interval between
the DAD and the subsequent stimulus, JSR is not reloaded sufficiently with Ca\(^{2+}\), and the resulting peak intracellular Ca\(^{2+}\) transient is not large enough to modulate \(I_{\text{Ca}}\) sufficiently and cause an EAD. This phenomenon is consistent with the observation of Capogrossi et al., who noted that spontaneous diastolic SR Ca\(^{2+}\) release limits [Ca\(^{2+}\)], potentiating the action potentials that display EADs in Fig 10. The reason that this action potential is not accompanied by a plateau EAD is the very high initial value of the \(I_k\) activation gate (X-gate=0.233, asterisk in Fig 9D). This generates a large outward \(I_k\) that, in spite of the fast decrease rate of [Ca\(^{2+}\)], results in a net outward (repolarizing) current that makes the occurrence of plateau EADs impossible.

**Triggered Activity Under Maintained Ca\(^{2+}\)-Overload Conditions**

When the capacity of \(I_{\text{NaCa}}\) is reduced (eg, by digitalis), the cell accumulates Na\(^+\) ions and, in turn, becomes overloaded with Ca\(^{2+}\) ions through the exchange mechanism of \(I_{\text{NaCa}}\) when it is paced at certain frequencies. If, in addition, \(\beta\)-adrenergic agonist (eg, isoproterenol) is applied, \(I_{\text{Ca}}\) is enhanced, and the Ca\(^{2+}\)-overload conditions are easily maintained. We simulate this situation by setting the following values: [Ca\(^{2+}\)]=3 mmol/L, resting [Ca\(^{2+}\)]=0.3 mmol/L, [Na\(^+\)]=15 mmol/L, 30% increase of \(P_{\text{Ca}}\), [CSQN]=82.5%, and \(G_{\text{CaCa}}=20\) mmol/L per millisecond. The threshold for the JSR release is increased to 82.5% to simulate the behavior of dog and guinea pig myocytes in which spontaneous Ca\(^{2+}\) release does not occur under normal conditions. Note that to simulate spontaneous release as a result of pacing under normal conditions (no Ca\(^{2+}\) overload), such as occurs in the rat, we previously set the JSR threshold at [CSQN]=70%. Under the conditions set above (ie, [CSQN]=82.5%), the cell maintains the overload condition but remains quiescent. We pace the cell under these conditions to investigate the possibility of triggered activity. As shown in Fig 11, the cell is paced with a train of eight stimuli at a BCL of 500 milliseconds. After the last paced beat, a triggered action potential is generated with a coupling interval of 1020 milliseconds (panel A). It is generated by spontaneous Ca\(^{2+}\) release from the SR (panels B and D), the same mechanism as we described previously for DADs. The subsequent triggered action potentials are elicited earlier and earlier (shorter coupling intervals) because of the increase of [Ca\(^{2+}\)]\(_{\text{SR}}\) (panel E). The rhythm becomes faster and faster until a dynamic steady state is reached. Note that the level of [Ca\(^{2+}\)]\(_{\text{SR}}\) after the pacing period (panel E) is always above the action potential threshold. Therefore, sustained triggered activity is maintained by spontaneous Ca\(^{2+}\) release. It takes \(\approx\)30 seconds to reach the steady state with a sustained rhythm at a BCL of 430 milliseconds and an APD of 310 milliseconds. Note that the behavior of \(I_{\text{NaCa}}\) and \(I_{\text{Ca}}\) (panels C and D) during pacing is very different from that during the triggered activity. During pacing (first eight beats), the membrane is depolarized to the plateau potential before the intracellular Ca\(^{2+}\) transient starts to increase, following the normal kinetics of the CICR process. \(I_{\text{NaCa}}\) and \(I_{\text{Ca}}\) are activated by an increase in [Ca\(^{2+}\)] at a time when the membrane potential is positive and display an early positive (outward) component. In contrast, during triggered activity, [Ca\(^{2+}\)] increases before the membrane is depolarized, since Ca\(^{2+}\) is released spontaneously from the overloaded JSR. As a result, \(I_{\text{NaCa}}\) and \(I_{\text{Ca}}\) are activated at a time when the membrane potential is still negative and display an early negative (inward) component (panels C and D). Once the triggered action potential reaches the plateau level while the intracellular Ca\(^{2+}\) transient is still large, both Ca\(^{2+}\)-activated currents display a positive (outward) component. This biphasic behavior of \(I_{\text{NaCa}}\) and \(I_{\text{Ca}}\) is only observed during triggered activity and not during pacing. Also, note that the inward component of \(I_{\text{NaCa}}\) is depressed by the increase of [Na\(^+\)] (15 mmol/L compared with the normal 10 mmol/L). Therefore, the peak inward current (-2.2 \(\mu\)A/\(\mu\)F) of the depressed \(I_{\text{NaCa}}\) is only about half that of \(I_{\text{Ca}}\) (-4 \(\mu\)A/\(\mu\)F) during triggered activity. This implies that \(I_{\text{Ca}}\) is much more important than \(I_{\text{NaCa}}\) in generating triggered activity under Na\(^+\)- and Ca\(^{2+}\)- overload conditions.
As indicated by arrows in Fig 11A, two consecutive plateau EADs appear on the first-paced and first-triggered action potentials. This was also observed experimentally by Priori and Corr18 when using the β-adrenergic agonist isoprenaline to enhance the Ca²⁺ inward current though the L-type Ca²⁺ channel (a situation that is simulated in our model by a 30% increase of the maximum channel conductance). In Fig 12, two action potentials are compared: one (the first paced action potential from Fig 11) with two plateau EADs and the other (the second paced action potential from Fig 11) with one plateau EAD. The ability to generate two consecutive EADs during an action potential is created by the self-inhibition of the Ca²⁺ channel due to the enlarged inward ICa caused by the channel agonist. At the double-EAD action potential shown in Fig 12, the fast decrease of the intracellular Ca²⁺ transient brings about a recovery of the ICa-gate and initiates the first EAD as described in the previous section. However, the inward ICa slows the rate of decrease of the intracellular Ca²⁺ transient (asterisk in panel B), resulting in a relatively small ICa (asterisk in panel C) on a background of fast increasing outward IK (asterisk in panel D). Therefore, the net membrane current changes from inward (depolarizing) to outward (repolarizing), causing the first EAD. The repolarization phase of the first EAD attenuates the increase of the outward IK (pound sign in panel D) at a time when the intracellular Ca²⁺ transient still decreases (pound sign in panel B). As a result, the ICa-gate is recovering and ICa is increasing faster than outward IK. This results in another phase of net inward (depolarizing) current that generates the second EAD. The initial value of the X-gate (ie, refractoriness) just before the stimulus is applied is much smaller at the double-EAD action potential than at the single-EAD action potential. This results in a long APD and creates the opportunity to generate a second phase of net inward current and a second EAD.

To evaluate the importance of INaCa in generating triggered activity under maintained overload conditions, we set INaCa = 0 at the time when the first spontaneous Ca²⁺ release from the overloaded junctional sarcoplasmic reticulum (JSR) occurs. The protocol and all other conditions are the same as in Fig 11. In Fig 13, only one DAD is observed after the last paced action potential (panel A). Subsequently, [Ca²⁺]JSR never reaches the threshold release level, and no additional episodes of spontaneous release are observed (panel F). For comparison, we adjust INaCa to compensate for the loss of INaCa in terms of total Ca²⁺-activated peak inward current. As shown in Fig 14, only one triggered action potential is obtained with the
enlarged \( I_{NaCa} \) (panel A). Then, the large-capacity \( I_{NaCa} \) (panel C) extrudes the Ca\(^{2+}\) ions that were loaded during pacing (see the fast decrease of \([Ca^{2+}]_{NSR}\) (panel E), and JSR never reaches the release threshold again (panel F). Note that the rate of decrease of \([Ca^{2+}]_{NSR}\) before \( I_{NaCa} \) enhancement (0.347 mmol/L per second, asterisk in panel E) is about one third the rate for the enhanced \( I_{NaCa} \) (pound sign in panel E). This demonstrates that with an enhanced \( I_{NaCa} \), Ca\(^{2+}\) overload is not easily maintained, and triggered activity is very unlikely to be sustained. A different method of enhancing \( I_{NaCa} \) is to decrease \([Na^{+}]\) from 15 to 10 mmol/L after the pacing period. As a result, only three triggered beats (not shown) are obtained even when \( I_{NaCa} \) is present, and the cell returns to a quiescent state. Therefore, Na\(^{+}\) overload that acts to depress \( I_{NaCa} \) is also an important factor in sustaining triggered activity.

**Effects of Overdrive on Ion Distribution**

Overdrive can modify the distribution of ions across the membrane and cause suppression of pacemaker cells.\(^{19}\) To simulate the redistribution of ions caused by overdrive, we pace the cell at a frequency of 2 Hz for 1 minute. The results are shown in Fig 15. Similar to the previous simulations, overdrive pacing loads the NSR with Ca\(^{2+}\) ions (panel A), bringing about an increase of the peak intracellular Ca\(^{2+}\) transient (panel B). The fluctuations in \([Ca^{2+}]_{NSR}\) are caused by Ca\(^{2+}\) translocation from the NSR to the JSR and by Ca\(^{2+}\) uptake by the NSR. After a 1-minute drive, \([Na^{+}]\) increases from 10 to 12.14 mmol/L (panel D), resulting in an increase of \( I_{NaK} \) from 0.28 to 0.34 \( \mu A/\mu F \) (panel C). This increase (0.06 \( \mu A/\mu F \)) of outward \( I_{NaK} \) is very small compared with \( I_{K} \) and \( I_{Ca} \), which are of the order of 1 to 4 \( \mu A/\mu F \) (Fig 17). Therefore, the effect of overdrive on the excitability of the cell membrane is negligible in ventricular cells. However, in pacemaker cells, the pacemaker current responsible for phase-4 depolarization is also very small; therefore, the small increase of outward \( I_{NaK} \) by overdrive could have a significant effect on membrane excitability, resulting in overdrive suppression. During the overdrive period, \([K^{+}]\) also decreases monotonically from 145 to 142.6 mmol/L. This could increase K\(^{+}\) accumulation in the extracellular clefts, bringing about an increase of the effective \([K^{+}]_{e}\) and a decrease of the maximum diastolic potential.\(^{19}\)

**Discussion**

**Postextrasystolic Potentiation**

As proposed by Yue et al.,\(^2\) the underlying mechanism of postextrasystolic potentiation can be explained by an excitation-contraction coupling model that includes two functional (or anatomic) compartments in the SR. In our model, SR is divided into an uptake compartment (NSR) and a release compartment (JSR), and Ca\(^{2+}\) ions are transferred from the NSR to the JSR by a translocation process. The time constant of Ca\(^{2+}\) translocation between NSR and JSR in the model is 180 milliseconds, close to the average value of 182±44 milliseconds measured by Yue et al. The upward shift and leftward shift of the postextrasystolic restitution curve in re-
sponse to shortening of the interval preceding the extrasystole \((T_{es})\) are well duplicated by the model (see Fig 3). The upward shift of the restitution curve (post-extrasystolic potentiation\(^3\)) is caused by the time delay associated with the translocation of Ca\(^{2+}\) from the NSR to the JSR. As shown in Fig 2C, if \(T_{es}\) is decreased by 500 milliseconds (asterisk with arrow) to 240 milliseconds (empty arrow), a smaller fraction of the Ca\(^{2+}\) content of the NSR is transferred to the JSR during \(T_{es}\). \([Ca^{2+}]_{JSR}\) is 37.25\% instead of 53.1\% and fewer Ca\(^{2+}\) ions are released during the extrasystole. As a result, a larger amount of Ca\(^{2+}\) ions remain in the NSR to be transferred from the NSR to the JSR during \(T_{rest}\). This, in turn, increases the amount of Ca\(^{2+}\) ions released from the JSR into the myoplasm during the postextrasystole \([Ca^{2+}]_{JSR}\) increases from 69.57\% to 70.1\%, and peak intracellular Ca\(^{2+}\) transient increases from 1.054 to 1.083 \(\mu\)mol/L, causing a stronger muscle contraction (postextrasystolic potentiation). The very small increase in \([Ca^{2+}]\), reflects both the short priming period (only four beats) and the buffering of myoplasmic calcium by calmodulin and troponin. The leftward shift of the restitution curve in response to a shorter \(T_{es}\) is caused by the shortening of the APD at the extrasystole, which results in a faster membrane recovery time and a shorter minimum \(T_{rest}\). Note that the leftward shift implies that the same \(\Delta[Ca^{2+}]\) occurs at a shorter \(T_{rest}\) for a shorter \(T_{es}\), consistent with the potentiation phenomenon. It should be mentioned that our model of Ca\(^{2+}\) translocation in SR does not depend on the state of the sarcolemma (membrane potential). Yue et al.\(^3\) proposed that such dependence exists and is responsible for the leftward shift of the restitution curve. However, our simulations reproduce this shift without the need to implicate any interaction between the state of the sarcolemma and the translocation of Ca\(^{2+}\) in SR.

By setting the translocation time constant at 180 milliseconds, the time constant of the simulated restitution curves (Fig 3) is 253±18 milliseconds, which differs from the 182±44 milliseconds measured by Yue et al.\(^3\). This could be due to additional delays that are introduced by other processes such as Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. During the excitation-contraction coupling cycle, the fluctuations of \([Ca^{2+}]_{JSR}\) are very small \((=0.2\) mmol/L, see Fig 2B). Therefore, the time course of return of released Ca\(^{2+}\) ions to the JSR is dominated by the time delay between NSR and JSR, although small delays are introduced by the release and uptake processes. Also, the time course of Ca\(^{2+}\) transfer between NSR and JSR is very different in different species. In ferret ventricular myocardium, Wier and Yue\(^4\) obtained a time constant >750 milliseconds for the restitution curves at 30°C, which differs greatly from the 182±44 milliseconds measured by Yue et al.\(^4\) in canine left ventricles at 36±1°C.

**DADs and Rhythmic Activity**

A DAD is defined as a depolarizing afterpotential that begins after normal repolarization is complete.\(^5\) We use the term “rhythmic activity” to describe repetitive activity that could be triggered or spontaneous.

In mammalian ventricular cells, at the postrepolarization phase of an action potential, \(I_{NaCa}\) is inactivated at a membrane potential of \(\approx -35\) mV (Fig 4 in our accompanying article\(^1\)), and \(I_{NaCa}\) is monotonically decreasing in response to the monotonic decrease of \([Ca^{2+}]\). At the same phase, the membrane is held at the resting potential by the high conductance of \(I_C\) (see Fig 17). Therefore, without the involvement of an intracellular event, the ventricular cell remains quiescent under normal or Ca\(^{2+}\)-overload conditions, unless an external stimulus is applied. However, the phenomenon of spontaneous muscle contraction has been observed universally in mammalian myocardium, including atria, Purkinje fibers, and ventricles,\(^1\) and also in skinned cardiac cell preparations.\(^12\) By monitoring the cell length to measure muscle contraction simultaneously with indo 1 measurements of intracellular Ca\(^{2+}\) transients, spontaneous muscle contraction was found to be associated with spontaneous Ca\(^{2+}\) release from the SR.\(^11,13,14\) Also, by rapid addition of caffeine to the bathing milieu of rat ventricular cells, Capogrossi et al.\(^13\) observed a train of repetitive spontaneous action potentials without the involvement of slow phase-4 depolarization that is indicative of pacemaker cells. In the experiments, this rhythmic activity lasts for 10 beats and is followed by DADs. This behavior is duplicated accurately by the simulations of Fig 9 under the condition of high-level Ca\(^{2+}\) overload. In the simulations, this rhythmic activity lasts for 11 beats and is also followed by DADs. The simulations support the hypothesis that the underlying mechanism of DADs and of spontaneous rhythmic activity in mammalian ventricular cells is spontaneous Ca\(^{2+}\) release from the overloaded JSR, which is triggered from “inside” the JSR membrane.\(^11\) When spontaneous Ca\(^{2+}\) release occurs, the intracellular Ca\(^{2+}\) transient reaches its peak value quickly and activates a transient inward current, \(I_{T}\).\(^20-25\) This, in turn, generates a DAD or an action potential that induces muscle contraction. Two different Ca\(^{2+}\)-activated membrane processes can potentially contribute to \(I_T\). These are \(I_{NaCa}\) (the Na\(^{+}\)-Ca\(^{2+}\) exchanger) and \(I_{NaCa}(Ca)\) (a nonspecific Ca\(^{2+}\)-activated channel). The relative contribution of these processes to \(I_T\) (and therefore their relative importance to arrhythmogenesis) is still a matter of controversy.\(^20-25\) Although it is experimentally difficult to separate these two components and study their individual contributions, this was achieved by the model simulations (see “Results”).

An important result is that the relative importance of \(I_{NaCa}\) and \(I_{NaCa}(Ca)\) depends on the degree of Ca\(^{2+}\) overload, as summarized below.

**Normal Conditions (No Ca\(^{2+}\) Overload)**

After pacing at a BCL of 400 milliseconds for 11 beats, two DADs of small amplitude (<2.6 mV), associated with two spontaneous Ca\(^{2+}\)-release episodes, are observed (see Fig 4). For this particular simulation (normal conditions), the threshold for spontaneous Ca\(^{2+}\) release from JSR was set at \([Ca^{2+}]_{JSR}=0.70\%\). This is a relatively low threshold that can trigger release with a slight increase of \([Ca^{2+}]_{JSR}\). Such high sensitivity is characteristic of ventricular cells from the rat but not from other mammals. As a result of pacing under normal conditions (normal resting \([Ca^{2+}]_{JSR}=0.12\ \mu\)mol/L, \([Ca^{2+}]_{JSR}\) increases to the threshold level, triggering a spontaneous release. As a result, the peak intracellular Ca\(^{2+}\) transient reaches 0.54 \(\mu\)mol/L, activating \(I_{NaCa}\) and \(I_{NaCa}(Ca)\) with inward amplitudes of 1.13 and 0.49 \(\mu\)A/\u2126F, respectively \((I_{NaCa}, I_{NaCa}(Ca)=2.3\)). We conclude, therefore, that under normal conditions, \(I_{NaCa}\) is the

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major component of \( I_T \) and is much more important than \( I_{Na(Ca)} \) to the generation of DADs. The low amplitude of \( I_{Na(Ca)} \) results from its Hill coefficient of 3 and its half-saturation \([Ca^{2+}]_s\) of 1.2 \( \mu \)mol/L (see Appendix 2 and the accompanying article). For these parameters, \( I_{Na(Ca)} \) is activated to only 1/12 its maximum at \([Ca^{2+}]_o=0.54 \mu \)mol/L, the peak intracellular \( Ca^{2+} \) transient produced by the spontaneous release under normal conditions.

**Minimum \( Ca^{2+} \) Overload**

By loading the cell to a level of \([Ca^{2+}] \) defined as minimum overload (ie, resting \([Ca^{2+}]_o=0.3 \mu \)mol/L, Ebara et al), a spontaneous action potential followed by a DAD is generated (Fig 5) by spontaneous \( Ca^{2+} \) release from the overloaded JSR (note \([CSQN]_m=70\%\)). The peak intracellular \( Ca^{2+} \) transient can reach a level of 1.6 \( \mu \)mol/L under these conditions, resulting in 70\% activation of \( I_{Na(Ca)} \). The amplitude of \( I_{Na(Ca)} \) is 3.4 \( \mu \)A/\( \mu \)F, 1.2 times larger than in normal conditions (Fig 5). Therefore, under conditions of minimum \( Ca^{2+} \) overload, both \( I_{Na(Ca)} \) and \( I_{Na(Ca)} \) are almost equally important in their contribution to \( I_T \) and to the generation of the spontaneous action potential.

**High Level of \( Ca^{2+} \) Overload**

Resting \([Ca^{2+}]_o\) is set at 0.4 \( \mu \)mol/L in this case. The simulation produces spontaneous rhythmic activity that lasts for 11 beats and is followed by DADs (see Fig 9). This simulated behavior was observed experimentally in rat ventricular cells after rapid addition of caffeine to the bathing milieu.\(^{13}\) The peak intracellular \( Ca^{2+} \) transient can reach 2.5 \( \mu \)mol/L, resulting in 90\% activation of \( I_{Na(Ca)} \). However, \( I_{Na(Ca)} \) is also increased by the high peak intracellular \( Ca^{2+} \) transient. Consequently, \( I_{Na(Ca)} \) reaches an amplitude of 4.4 \( \mu \)A/\( \mu \)F and is only 7\% larger than \( I_{Na(Ca)} \). Therefore, under high-level \( Ca^{2+} \) overload, both \( I_{Na(Ca)} \) and \( I_{Na(Ca)} \) are equally important in generating spontaneous rhythmic activity. It should be noted that the foot of the action potentials generated by spontaneous \( Ca^{2+} \) release in the simulations (eg, Figs 5 and 11) displays a faster rising time than that observed in the experiments.\(^{13}\) This is partly because the release of \( Ca^{2+} \) by the JSR in the model occurs at a unifocal site and with a fixed threshold. In the real situation, the cell contains over 50 JSR release sites, and the release threshold is statistically distributed.\(^{13,14}\) Therefore, in contrast to the model behavior, not all the release sites release their \( Ca^{2+} \) contents simultaneously. Also, different \( Ca^{2+} \) diffusion times from the multiple release sites to the sarclemma act to desynchronize activation of the transient inward current. These two dispersion effects (nonsimultaneous multifold release sites and \( Ca^{2+} \) diffusion) act to make the activation process more gradual, resulting in a slower action potential foot. It should be emphasized that although the exact time course of the foot and details of \( I_T \) that depend on the degree of spatial dispersion are not reproduced by the model, the simulations duplicate the basic phenomena of DADs and spontaneous activity under a variety of conditions. Spatial nonuniformity of \( Ca^{2+} \) release from the SR is not required for the generation of a transient inward current and DADs. As stated in Berlin et al:\(^ {25}\) “The increase in \([Ca]\), that underlies \( I_T \) can occur simultaneously in several areas of the cell and lead to an apparent synchronization of electrical and mechanical activity. This result suggests that \( I_T \) can occur with complete synchronization of \( Ca^{2+} \) release (ie, no wave-like propagation of increased \([Ca]\) ).”

**\( Na^{+} \) and \( Ca^{2+} \) Overload**

When \( I_{Na(Ca)} \) is depressed (eg, by application of digitalis or under conditions of \([K^{+}]_o=0 \) mmol/L), \( Na^{+} \) accumulates in the cell and attenuates \( Ca^{2+} \) extrusion from the cell through the exchange process, \( I_{Na(Ca)} \). As a result, the cell becomes overloaded with both \( Na^{+} \) and \( Ca^{2+} \). Based on the kinetics of \( I_{Na(Ca)} \), the increase of \([Na^{+}]_o\) attenuates both the \( Ca^{2+} \)-efflux mode and the inward amplitude of \( I_{Na(Ca)} \). Therefore, the \( Ca^{2+} \) overload is easily maintained when \( Na^{+} \) overload is present. In the simulations (Fig 11), \([Na]_o\) increases from the normal value of 10 to 15 mmol/L, and resting \([Ca^{2+}]_o\) is 0.3 \( \mu \)mol/L. In addition, \([CSQN]_m\) is set at 82.5\%. This relatively large threshold for release simulates stable SR characteristics in the dog and guinea pig, in contrast to the “quick release” SR of the rat ([CSQN]_m=70\% in our simulations). Under these conditions, the cell remains quiescent, and the overload condition is maintained. After pacing at 2 Hz for eight beats, sustained triggered activity is obtained (Fig 11). During sustained triggered activity, \( I_{Na(Ca)} \) has an amplitude of 4 \( \mu \)A/\( \mu \)F, which is 1.8 times larger than \( I_{Na(Ca)} \). Therefore, under \( Na^{+} \)- and \( Ca^{2+} \)-overload conditions, \( I_{Na(Ca)} \) is the major component of \( I_T \) and is more important than \( I_{Na(Ca)} \) to the generation of sustained triggered activity. It should be noted that the triggered activity lasts for only three beats when \([Na]_o\) is reduced from 15 mmol/L to its normal value of 10 mmol/L and for only one beat when \( I_{Na(Ca)} \) is increased threefold and \( I_{Na(Ca)} \) is set to zero (Fig 14). This implies that triggered activity can be sustained under the conditions of depressed \( I_{Na(Ca)} \). An augmented \( I_{Na(Ca)} \) can generate triggered activity in the absence of \( I_{Na(Ca)} \), but this triggered activity cannot be sustained. It should be added that DADs can also be induced by blocking the \( Na^{+} \)-K+ pump directly in the model, simulating this effect of cardiotonic steroids (“digitalis toxicity”). An example is shown in Fig 16, where DADs appear after a train of paced action potentials under \( Na^{+} \)-K+ block (panel A) but not under control conditions (panel B). The DADs are associated with spontaneous \( Ca^{2+} \) release from the SR (see \([Ca^{2+}]_i \) tracings) and corresponding \( I_T \) (see transmembrane current tracings). The simulated behavior is consistent with the experimental behavior for a similar protocol,\(^ {25}\) in which the \( Na^{+} \)-K+ pump was suppressed by exposure to ouabain or strophanthidin.

**EADs**

An EAD is defined as a depolarizing afterpotential that begins before the completion of repolarization of an action potential and causes an interruption or retardation of normal repolarization. According to Cranefield and Aronson,\(^ {5}\) EAD should only be used to describe an afterpotential for which the net membrane current remains outward or is zero. An EAD that fulfills these requirements is simulated in Fig 17. Two action potentials are shown (panel A), one for \([K^{+}]_o=5.4 \) mmol/L (normal concentration) and one for \([K^{+}]_o=2 \) mmol/L (hypokalemia). The major ionic currents (except the \( Na^{+} \) current \( I_{Na} \), which does not contribute to the repolarization phase) are plotted for both conditions from panel B to panel E. As explained previously,\(^ {10}\) \( I_T \) dominates the
changes in the rate of repolarization caused by changes in [K\(^+\)]\(_o\) (peaks indicated by full arrows in panel D). For the action potential of [K\(^+\)]\(_o\)=2 mmol/L, a reduced net outward current at the late repolarization phase induces a hump in the action potential (panel A, empty arrow), which qualifies as an EAD according to the definition of Cranefield and Aronson.\(^5\) The H coefficient\(^2\) of this EAD is \(\approx\)50%. The H coefficient is designed to measure the reduction in slope of the membrane potential at the hump. H is zero if the slope is normal and an EAD does not occur. In Fig 17, \(I_{NaCa}\) (panel B) is inactivated at a membrane potential of \(\leq-35\) mV and does not contribute to the EAD. The inward \(I_{NaCa}\) (panel E) supports the EAD against the increase of the outward \(I_V\) that is caused by its negative slope (panel D) and against the outward (but decreasing) \(I_K\). Since \(I_K\) dominates changes in the repolarization rate of the action potential in response to changes in [K\(^+\)]\(_o\), we plot its components (\(I_{K1}\), \(I_{Kp}\), and \(I_{NaK}\)) that are influenced by [K\(^+\)]\(_o\) (Fig 18). Clearly, at the time of the EAD (empty arrow), \(I_V\) (panel A) is almost completely determined by \(I_{K1}\) (panel B), and contributions from \(I_{NaK}\) and \(I_{Ko}\) can be neglected. Therefore, the occurrence and shape of an EAD at low [K\(^+\)]\(_o\) are determined by the balance of \(I_{K1}\), \(I_{K1}\), and \(I_{NaCa}\) during the late repolarization phase of an action potential.

As shown in “Results,” two types of EADs that are not included in the Cranefield and Aronson definition appear in our simulations. These EADs are associated with a net inward current that results in depolarization, rather than retarded repolarization, of the membrane potential. A “plateau EAD” appears at the plateau phase or early repolarization phase of an action potential (Figs 5 and 8 through 14). Plateau EADs were observed experimentally by Priori and Corr\(^18\) in the presence of a β-adrenergic agonist (isoproterenol), by Marban et al\(^19\) during exposure to cesium chloride, and by January and Riddle\(^29\) in the presence of the L-type Ca\(^{2+}\) channel agonist Bay K 8644. Since in their study the EADs were eliminated by ryanodine, Priori and Corr concluded that Ca\(^{2+}\) release by the SR is a key process in the genesis of plateau EADs. In contrast, Marban et al demonstrated that plateau EADs were not suppressed by ryanodine or by loading with intracellular Ca\(^{2+}\) chelators. Moreover, Bay K 8644 potentiated EADs, whereas nitrendipine (Ca\(^{2+}\) channel antagonist) abolished EADs. They concluded that EADs do not...
activate voltage-dependent X-gate (Figs 11 and 12). Similar oscillatory responses at the plateau phase were observed in hypertrophied rat papillary muscle. It should be emphasized that this type of EAD is generated by the kinetics of ICa and IK. Ca2+ release by the SR (induced from "outside" the SR by the increase of [Ca2+]) influences this process through its effect on ICa (Ca2+ inactivation through fCa). This is very different from the mechanism of DADs, which result from the activation of INaCa and INaK by spontaneous Ca2+ release from the SR, triggered from "inside" the SR by Ca2+ overload. Our theoretical finding that plateau EADs and DADs do not share a common mechanism is consistent with the experimental observations of Marban et al. In their experiments, ryanodine and loading with Ca2+ chelators suppressed DADs but not EADs, clearly indicating that SR Ca2+ release is the basis for DAD generation but not for EAD generation. As stated above, their experiments using Ca2+ channel agonists and antagonists suggested that ICa, and not SR release, underlies the generation of plateau EADs. The different mechanisms of plateau EADs and of DADs elucidated by the simulations presented here are consistent with these experimental findings and with the experimental observations of Janu and Riddle. They contradict, however, the conclusion of Priori and Corr that DADs and EADs share a common mechanism that involves Ca2+ release from the SR, enhanced INaCa, and activation of INaK. It is interesting to note that, similar to experimental findings, plateau EADs and DADs can be generated in the same simulated experiment (eg, see Fig 5), under the same set of conditions, even though their underlying mechanisms are very different.

The other type of EAD that is not included in the definition of Cranefield and Aronson is Ca2+ entry through sarcolemmal slow Ca2+ channels. Similarly, by using Ca2+ channel blockers to eliminate the EADs, Janu and Riddle concluded that recovery from inactivation and reactivation of the L-type Ca2+ channel are key processes to the generation of plateau EADs. The conclusions of Marban et al. and Janu and Riddle are supported by our simulations. The simulations demonstrate that the sufficiently fast decrease of the intracellular Ca2+ transient makes possible the recovery of ICa during the action potential plateau through the recovery of the Ca2+-dependent inactivation gate, fCa (Figs 5 and 10). At the same time, the f-gate also recovers after its voltage-dependent characteristics. The recovered inward ICa changes the direction of the net membrane current from outward to inward, resulting in depolarization of the membrane. The increase of membrane potential reactivates ICa through the voltage-dependent activation d-gate. It also continues to activate IK through the voltage-dependent activation X-gate (Figs 10 and 12). Since the kinetics of the X-gate is much slower than that of the d-gate, the membrane potential depolarizes first to a peak value because of the increase of ICa and then repolarizes as IK increases. This sequence of events generates the plateau EAD. In certain simulations (Figs 11 and 12), two consecutive plateau EADs during a single action potential are observed. A similar phenomenon was also observed experimentally by Priori and Corr (their Fig 7A). As clarified by the simulations, this behavior results from the interaction between the process generating the plateau EAD and the self-inhibition of the recovery of ICa through the Ca2+-dependent inactivation gate (fCa) that results from the large inward ICa (see Fig 12).
result of the outward currents that are activated by spontaneous Ca\(^{2+}\) release.

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**Appendix 1: Equations**

I. Ionic currents in the sarcolemma

a. Ca\(^{2+}\) currents: \(I_{Ca}=I_{Ca}+I_{Ca,K}+I_{Ca,Na}\);

\[
I_{Ca}=d \cdot f \cdot f_{Ca} \cdot I_{Ca} \quad \text{and} \quad I_{Ca,Na}=d \cdot f \cdot f_{Ca} \cdot I_{Ca,Na}
\]

For ion S, including Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\):

\[
I_{S} = P_{S} \cdot \left[ S \right] \cdot \exp(z_{S}VF/RT) - \gamma_{o} \cdot \left[ S \right] \cdot \exp(z_{S}VF/RT) - 1
\]

\[
P_{Ca} = 5.4 \times 10^{-4} \text{ cm/s; } \gamma_{Ca} = 1; \quad \gamma_{Ca} = 0.341;
\]

\[
P_{Na} = 6.75 \times 10^{-7} \text{ cm/s; } \gamma_{Na} = \gamma_{Na} = 0.75;
\]

\[
P_{K} = 1.93 \times 10^{-7} \text{ cm/s; } \gamma_{K} = \gamma_{K} = 0.75;
\]

\[
f_{Ca} = 1/[1+([Ca^{2+}]_{i}/K_{m,Ca})]; \quad K_{m,Ca} = 0.6 \mu mol/L;
\]

\[
d_{i} = 1/[1+\exp(-(V+10)/6.24)];
\]

\[
f_{i} = 1/[1+\exp((V+32)/8)] + 0.6/[1+\exp((50-V)/20)];
\]

\[
f = 1/[1+\exp(-[0.0337 \cdot (V+10)])] + 0.02;
\]

\[
\alpha = \tau_{i}/\tau_{o} \quad \beta = (1-\tau_{i})/\tau_{o}
\]

b. Na\(^{+}-Ca^{2+}\) exchanger: \(I_{NaCa}\)

\[
I_{NaCa} = \frac{k_{NaCa}}{K_{m,Na}+[Na^{+}]_{o}} \cdot \frac{1}{K_{m,Na}+[Ca^{2+}]_{o}} \cdot \frac{1}{1+k_{NaCa} \cdot \exp[(\eta-1) \cdot V \cdot F/RT]} \cdot \exp(\eta \cdot V \cdot F/RT)
\]

\[
[Na^{+}]_{o} \cdot [Ca^{2+}]_{o} \cdot \exp(-[\eta-1] \cdot V \cdot F/RT) \cdot [Na^{+}]_{o} \cdot [Ca^{2+}]_{o};
\]

\[k_{NaCa} = 200 \mu A/\mu F; \quad K_{m,Na} = 87.5 \text{ mmol/L; } K_{m,Na} = 1.38 \text{ mmol/L; } k_{NaCa} = 0.1 \text{ and } \eta = 0.35.
\]

c. Nonspecific Ca\(^{2+}\)-activated current: \(I_{Ca}(Ca)\)

\[
I_{Ca}(Ca) = \frac{1}{1+(K_{m,Na}/[Ca^{2+}]^{3})}
\]

\[
I_{Ca}(Na_{o}) = \frac{1}{1+(K_{m,Na}/[Ca^{2+}]^{3})};
\]

\[
I_{Ca}(Ca) = I_{Ca,K} + I_{Ca,Na} \quad \text{and} \quad K_{m,Na} = 1.75 \times 10^{-7} \text{ cm/s; } K_{m,Na} = 1.2 \mu mol/L; \quad \text{and}
\]

\[
E_{Ca}(Ca) = \frac{[K^{+}]+[Na^{+}]}{F} \cdot K_{m,Na} + [Na^{+}]_{o};
\]

\(I_{Ca}(Ca)\) is computed from \(P_{Na}\) using the relation in I.a of this appendix.

d. Total time-independent current: \(I_{V}\)

\[
I_{V} = I_{Ca} + I_{Na} + I_{Ca,b} + I_{Na,b} + I_{Na,K}
\]

II. Ca\(^{2+}\) buffers in the myoplasm

Troponin (TRPN) and calmodulin (CMDN); buffered [TRPN] = [TRPN] - [Ca\(^{2+}\)]/[K\(^{+}\) + [Ca\(^{2+}\)]]; buffered [CMDN] = [CMDN] - [Ca\(^{2+}\)]/[K\(^{+}\) + [Ca\(^{2+}\)]];

\[TRPN = 70 \mu mol/L; \quad CMDN = 50 \mu mol/L; \quad K_{m,TRPN} = 0.5 \mu mol/L; \quad K_{m,TRPN} = 2.38 \mu mol/L.
\]

III. Ca\(^{2+}\) fluxes in the SR

a. Ca\(^{2+}\)-induced Ca\(^{2+}\) release of JSR

\[I_{rel} = G_{rel} \cdot ([Ca^{2+}]_{JSR} - [Ca^{2+}]_{i}) \text{ mmol/L per millisecond.}
\]

If \[\Delta([Ca^{2+}]_{i}) > \Delta([Ca^{2+}]_{i}) \text{ 2 milliseconds after the time of } \dot{V}_{ma},\]
Appendix 2: Definition of Symbols

V: membrane potential, mV
V′: time derivative of V, mV/ ms
Vmax: maximum rate of rise of V, mV/ ms
Km: half-saturation concentration of channel i, mmol/L
Ii: maximum current through channel i, µA/ µF
G: maximum conductance of channel i, mS/ µF
PA: permeability ratio of ion A to ion B
Ea: reversal potential of ion A, mV
Ea,N: Nernst potential of ion A, mV
αA: activity coefficient of ion A
zA: valence of ion A
[A]+, [A]: extracellular and intracellular concentrations of ion A, respectively, mmol/L
Ica: Ca2+ current through the L-type Ca2+ channel, µA/ µF
Icai: Na+ current through the L-type Ca2+ channel, µA/ µF
IKCa: K+ current through the L-type Ca2+ channel, µA/ µF
IcaL: total current (Icak + ICAK + ICAK) through the L-type Ca2+ channel, µA/ µF
d-gate: f-gate: activation gate and (voltage-dependent) inactivation gate, respectively, of the L-type Ca2+ channel
IcaL: Ca2+-dependent inactivation gate of the L-type Ca2+ channel
IcaL: Na+-Ca2+ exchanger, µA/ µF
kNCa: scaling factor of INaCa, µA/ µF
kmax: saturation factor of INaCa, at very negative potentials
η: position of the energy barrier controlling voltage dependence of INaCa
IiNCa: non-specific Ca2+-activated current (INaCa=INaNCa+INaK), µA/ µF
INaNCa: Na+ current through the non-specific Ca2+-activated channel, µA/ µF
IKCa: K+ current through the non-specific Ca2+-activated channel, µA/ µF
Ii: transient inward current (Ii=INaCa+INaK), µA/ µF
Ii: total time-independent current (Ii=INaCa+Ii)+INaK, µA/ µF
IK: time-independent K+ current, µA/ µF
IK: plateau K+ current, µA/ µF
INaK: Na+-K+ pump, µA/ µF
Ii(Ca): Ca2+ pump in the sarcolemma, µA/ µF
Ii(Ca): Ca2+ background leakage current, µA/ µF
INaB: Na+ background leakage current, µA/ µF
Ii(Ca): Ca2+ release from JSR to myoplasm, mmol/L per millisecond
Grel: rate constant of Ca2+ release from JSR, ms
(t) responses of activating (on) and deactivating (off) Ca2+ release from JSR, millisecond
Ii: Ca2+ uptake from myoplasm to JSR, mmol/L per millisecond
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