Early afterdepolarizations in cardiac myocytes: beyond reduced repolarization reserve

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Abstract

Early afterdepolarizations (EADs) are secondary voltage depolarizations during the repolarizing phase of the action potential, which can cause lethal cardiac arrhythmias. The occurrence of EADs requires a reduction in outward current and/or an increase in inward current, a condition called reduced repolarization reserve. However, this generalized condition is not sufficient for EAD genesis and does not explain the voltage oscillations manifesting as EADs. Here, we summarize recent progress that uses dynamical theory to build on and advance our understanding of EADs beyond the concept of repolarization reserve, towards the goal of developing a holistic and integrative view of EADs and their role in arrhythmogenesis. We first introduce concepts from nonlinear dynamics that are relevant to EADs, namely, Hopf bifurcation leading to oscillations and basin of attraction of an equilibrium or oscillatory state. We then present a theory of phase-3 EADs in nonlinear dynamics, which includes the formation of quasi-equilibrium states at the plateau voltage, their stabilities, and the bifurcations leading to and terminating the oscillations. This theory shows that the L-type calcium channel plays a unique role in causing the nonlinear dynamical behaviours necessary for EADs. We also summarize different mechanisms of phase-3 EADs. Based on the dynamical theory, we discuss the roles of each of the major ionic currents in the genesis of EADs, and potential therapeutic targets.

Keywords

Early afterdepolarizations • Repolarization reserve • Nonlinear dynamics • Oscillation • Arrhythmias

1. Introduction

Early afterdepolarizations (EADs) are secondary voltage depolarizations during the repolarizing phase of the cardiac action potential (AP). They are often associated with arrhythmias such as Torsade de Points (TdP) in the setting of cardiac diseases, including acquired and congenital long QT syndromes and heart failure. EADs were identified more than a half century ago originally called low-membrane potential oscillations in Purkinje cells (see Hauswirth et al. and early experimental references therein), with the term EADs coined later by Cranefield, and have been the subject of many experimental and computational studies from which important mechanistic insights have been gained. Experimental studies from the 1980s–14 led to the important conclusion that EADs occur when outward currents are reduced and/or inward currents are increased, resulting in the lengthening of action-potential duration (APD), with the recognition of the window calcium (Ca) current playing an important role. Under these conditions, the L-type Ca channel (I_{Ca,L}) may reactivate and reverse repolarization during the AP plateau. In addition, studies also have shown that intracellular Ca cycling, especially spontaneous Ca release, can be a cause of EADs. To account for the differential susceptibility of patients treated by anti-arrhythmic drugs to QT prolongation and TdP, Roden proposed the concept of ‘reduced repolarization reserve’ as a general condition promoting EAD formation, such that patients with reduced repolarization reserve due to genetic defects or other cardiac diseases were at a considerably higher risk of TdP when administered drugs that block K currents such as I_{Kr}. However, lengthening APD by increasing inward currents or reducing outward currents does not always cause EADs. Conversely, some drugs (e.g. isoproterenol) may cause EADs without significantly prolonging APD. Hondeghem et al. astutely observed that ‘triangulation’ of the AP plateau, rather than APD prolongation per se, is more likely to result in EADs and TdP, which narrows the conditions for EAD genesis. In recent studies, utilizing dynamical theory, we have developed a holistic view of how EADs arise and generate arrhythmias. The dynamical theory of EADs not only clarifies the mechanistic roles of repolarization reserve and AP triangulation, but also advances our current understanding of EADs beyond these concepts and provides

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insights into potential effective therapeutic targets. Key to the dynamics approach is the recognition that EADs are not simply depolarizations, but are oscillations in membrane voltage which eventually cease, allowing the myocyte to repolarize (Figure 1). The amplitude of the oscillations (EADs) varies with time, and, in many cases, the last EAD before full repolarization has the largest amplitude (Figure 1). To fully understand EADs, these dynamics need to be taken into consideration.

In this review, we summarize recent progress and the insights from the nonlinear dynamics analysis of EADs. We first introduce concepts from nonlinear dynamics that are relevant to EADs. We then present our recent nonlinear dynamics theory of EADs. Finally, we use this theory as a general framework to provide an integrative and holistic overview of the roles of the major ionic currents in the genesis of EADs. Potential therapeutic targets based on the dynamics analysis are also discussed at the end.

2. Nonlinear dynamics relevant to EADs

2.1 Bifurcation and oscillation

Oscillations are a ubiquitous phenomenon in biological systems, and in the heart include pacemaking of sino-atrial nodal (SAN) cells, automatically in the ventricles, and EADs in an AP. Oscillations are a nonlinear dynamical phenomenon, arising from a type of qualitative change, or bifurcation, called a Hopf bifurcation (Figure 2A).34 Up until the bifurcation point, the steady state (or equilibrium state) is stable. This means that starting from an initial state away from the steady state, the system may exhibit a transient oscillation, but will eventually reach the steady state (illustrated as the spiraling-in trace in Figure 2A). After the bifurcation point, however, the steady state becomes unstable, and the system can no longer remain at this state, but will go to the oscillatory state (illustrated as the spiralling-out trace in Figure 2A). For example, a ventricular myocyte’s oscillatory depolarizations or an SAN cell’s onset of pacemaking can be modelled mathematically as a Hopf bifurcation. The common biological processes causing oscillatory behaviours are a feedback loop with a steeply sloped nonlinear function; oscillations result when the parameters of the system are such that the equilibrium state is located in the steep region of this function. For a more comprehensive understanding, we present a simple biochemical reaction model to demonstrate the biological properties that cause Hopf bifurcations and oscillations in the online supplement (see Supplementary material online, Figure S1 and text).

2.2 Basin of attraction

When a system has only one stable solution, the system will always approach this state when starting from any initial state. In nonlinear systems, however, there can be more than one stable solution for the same set of parameters. Repolarization failure is an example. For instance, in a ventricular myocyte with large enough \( I_{Ca,L} \) conductance (or small enough K conductance), the AP can fail to repolarize. In this case, the myocyte voltage has two stable solutions: the resting state and a depolarized state. When the initial voltage is close to the resting potential, the myocyte stabilizes at the resting voltage. If a stimulus brings the voltage above the threshold for Na current activation, the myocyte’s voltage overshoots and then, by activating the excessive Ca current, stabilizes in a depolarized state without repolarizing. This is typical bistable behaviour of a nonlinear system, and the final state of the system depends on the initial conditions (Figure 2B), i.e. the initial values of voltage and other variables. The region of state space (the space spanned by all the variables) that contains all the initial conditions that lead to the same state is called the basin of attraction of that state. We will show here how basins of attraction relate to EAD genesis.
3. A nonlinear dynamics theory of EADs

3.1 Quasi-equilibrium states

To study AP repolarization, Noble et al.\textsuperscript{35,36} used a quasi-instantaneous (here called quasi-steady state) current ($I_{\text{QSS}}$) approach. In this approach, fast currents or gating variables are assumed to have reached steady states such that a slow variable, such as $I_{\text{k}}$ activation, can be used as a parameter to analyse the repolarization behaviours. Figure 3A shows the quasi-steady state $I$–$V$ curves from the LR1 myocyte model\textsuperscript{29,37} in which the activation gating variable $x$ of the time-dependent K current is set as a parameter. When $x=0$, there are three voltages (circles in Figure 3A) at which $I_{\text{QSS}}=0$. We call these voltages quasi-equilibrium states (QESs). The QES between $-90$ mV and $-80$ mV is the resting potential (labelled as ‘r’). As $x$ increases, the quasi-steady state $I$–$V$ curve moves upward (increasing outward current), and when $x$ becomes large, the upper two QESs (labelled as ‘s’ and ‘p’) disappear and only the r-state remains.

The existence of the two QESs (s- and p-states) at the plateau voltage is a result of the balance between the quasi-steady-state outward and inward currents. The quasi-steady-state inward currents, including window and pedestal $I_{\text{Ca,L}}$, window and late $I_{\text{Na}}$, and $I_{\text{NCX}}$ etc., are critical for counterbalancing the K currents to maintain the plateau QESs. For example, if one shifts the $I_{\text{Ca,L}}$ steady-state inactivation (SSI) curve for more negative voltages or the steady-state activation (SSA) curve to more positive voltages to reduce or eliminate the $I_{\text{Ca,L}}$ window current, the QES at the plateau voltage can be eliminated (Supplementary material online, Figure S2).\textsuperscript{33} Note that the transient components of $I_{\text{Na}}$ and $I_{\text{Ca,L}}$ do not contribute to the formation of the plateau QESs since they inactivate very rapidly, and in a normal AP in which the outward K currents are large and/or the quasi-steady-state inward currents are small, the QESs at the plateau may not exist.

3.2 Stability of the quasi-equilibrium states

The existence of the QESs at the plateau voltage (namely the p-state) is required for EAD formation, such that as the voltage approaches the p-state during the early repolarization phase, it spirals around the p-state producing the voltage oscillations that manifest as EADs (Figure 3B). However, whether oscillations occur or not depends on the stability of the p-state. If the p-state is stable, no oscillations can occur, but it can result in an ultralong APD without EADs\textsuperscript{33} If the p-state becomes unstable via a Hopf bifurcation as in Figure 2A, voltage oscillations can occur. A necessary property for the QES to become unstable is a steep slope of the SSA curve of $I_{\text{Ca,L}}$. The SSA curve plays the equivalent role of $f(x)$ in the chemical oscillations shown in the online supplement (see Supplementary material online, Figure S1) because it is a steeply increasing sigmoidal function that facilitates positive feedback, in which an increase in voltage causes more Ca channel openings to further elevate voltage. Oscillations only occur when the QES forms at the voltage range where the slope of the SSA curve is steep, which occurs normally between $-40$ mV and $-10$ mV, and thereby defines the window-voltage range for EADs (the grey zone in Figure 7B).

The stability of a QES depends not only on the slope of the SSA curve of $I_{\text{Ca,L}}$ but also the conductance and kinetics of all other ionic currents that contribute to its formation.\textsuperscript{29} For example, the stability of the p-state depends on the activation of the slow K current, i.e. as the slow K current activation increases, the QES becomes unstable via a Hopf bifurcation, leading to oscillations. The oscillations terminate at another bifurcation called a homoclinic bifurcation (see Supplementary material online, Figure S3).

3.3 EAD genesis

The existence of an unstable QES does not guarantee EADs, which also depend on other conditions. As discussed in Section 2.2, for a nonlinear system with multiple solutions, the state to which the system equilibrates depends on the initial conditions (the basin of attraction). Therefore, for an EAD to occur requires not only that the voltage is in the window range, but also that other variables, such as the gating variables of the K currents, are in a properly balanced range. As shown in the bifurcation diagram (see Supplementary material online, Figure S3), the oscillations occur only if the activation gating variable $x$ is in its critical range at the same time that voltage is in the window range. Therefore, both $x$ and $V$ need to enter their proper ranges to allow the oscillations to ocurrence.
achieved, for example, by adding a transient outward current to the repolarization speed (i.e. lowering the plateau more rapidly) can also grow slower and thus zones at roughly the same time, resulting in EADs. Also note that the window range, however, does not allow a lower take-off potential, in the voltage range of phase-3 of the AP. Most isolated myocyte studies have recorded phase-3 EADs, but some have shown phase-3 EADs. In a study by Guo et al., phase-3 EADs were recorded from isolated rabbit ventricular myocytes after exposure to the K⁺ blocker dofetilide (Figure 6A). In a computer model of rabbit ventricular myocytes, we were able to induce phase-3 EADs (Figure 6B), similar to the ones recorded in experiments by Guo et al. In our computer model, this requires a current such as I\textsubscript{NCX} to generate an inward current at more negative potentials, so as to oppose full repolarization and allow a lower take-off potential. However, the underlying dynamical mechanism is still the same as for phase-2 EADs discussed in Section 3.

EADs may occur at even lower take-off potentials, called late phase-3 EADs. To our knowledge, late phase-3 EADs have been recorded from intact tissue, but not from isolated myocytes. The postulated mechanism is the Ca transient outlasting the APD, causing I\textsubscript{NCX} and other Ca-activated inward currents to depolarize membrane voltage to the I\textsubscript{Na} threshold, a mechanism similar to that for delayed afterdepolarizations (DADs). However, whereas DADs are induced via spontaneous SR Ca release during diastole, late phase-3 EADs usually occur when the APD is substantially abbreviated such that the Ca transient outlasts repolarization. For example, recent studies showed that shortening APD, by activating the Ca-activated apamin-sensitive K activation mechanism is still the same as for delayed afterdepolarizations (DADs). However, whereas DADs are induced via spontaneous SR Ca release during diastole, late phase-3 EADs usually occur when the APD is substantially abbreviated such that the Ca transient outlasts repolarization. For example, recent studies showed that shortening APD, by activating the Ca-activated apamin-sensitive K

Figure 4 EAD genesis. (A) Voltage and x vs. t for an AP without EADs from the LR1 model. The parameters are the same as in Supplementary material online, Figure S3. (B) Same as (A) but the activation of I\textsubscript{K} was slowed. (C) Same as (A) but with I\textsubscript{Na} present to accelerate the early voltage decline. The scales of V and x were adjusted so that the window-voltage range and the critical range of x are in the same range of the plot (the grey zone).

4. Phase-3 EADs

The EADs shown in Figure 1 represent phase-2 EADs whose amplitudes are relatively small because the take-off potential is in the plateau voltage range.

The nonlinear dynamical theory of EADs is demonstrated in a number of experimental studies. Namely, reducing I\textsubscript{Ca,L} window current by shifting the SSA or SSI curves, which eliminates the QESs in the plateau phase, suppresses EADs induced by either H₂O₂ or hypokalaemia (Figure 5A) in rabbit ventricular myocytes. Blocking I\textsubscript{Na} by 4-AP eliminates H₂O₂-induced EADs in isolated rabbit ventricular myocytes (Figure 5B), which is explained by the failure to reach the basin of attraction of oscillations as shown in Figure 4. Many experimental recordings show that EAD amplitude gradually increases, with the last EAD being the largest (e.g. Figure 1), which is a consequence of the dual Hopf-homoclinic bifurcation scenario. In addition, EAD bursts induced by BayK8644 and isoproterenol in cultured rat ventricular myocyte monolayers exhibit the property of a decreasing frequency of the oscillations (Figure 5C), which is a classic signature of the Hopf-homoclinic bifurcation mechanism. This feature can be seen in the EADs in Figure 1. However, it is possible for EADs to occur without the Hopf-Homoclinic bifurcation if the QES (p-state) remains in the pre-Hopf bifurcation state. Under these conditions, transient oscillations can occur (see Figure 2A), manifesting as EADs. However, there will be only a few oscillations with damping amplitudes, which has been also observed in many experimental recordings. The homoclinic bifurcation causes EAD behaviour to be chaotic under periodic pacing, which explains the irregular beat-to-beat pattern of EADs that is widely observed in experiments. Since chaotic EADs facilitate arrhythmia initiation in tissue, this may provide mechanistic insight into why short-term QT variability, more so than the QT interval per se, is predictive of TdP.
current in heart failure or by activating $I_{\text{KATP}}$, caused late phase-3 EADs to initiate ventricular fibrillation.

Rather than resulting from a primarily cellular origin, a phase-3 EAD may also result from electrotonic coupling between regions with and without phase-2 EADs in heterogeneous tissue. In a recent study, Maruyama et al. showed that under the condition of low $[K]_o$ and $I_{\text{Kr}}$ blockade, phase-3 EADs occurred in heterogeneous regions in rabbit hearts, which was also demonstrated in computer simulations. This is a novel mechanism of phase-3 EAD formation which only occurs at the tissue scale.

5. An integrative overview of the roles of ionic currents in EAD genesis

Genetic mutations, drugs, ionic imbalances, remodelling etc., result in alterations in ion currents and Ca cycling which can promote EADs (Figure 7A). Traditionally, the effects of ion currents on EADs have been interpreted in the context of reduced repolarization reserve (dashed arrows in Figure 7A), which is not a sufficient condition for EADs. This concept can now be clarified: using the dynamics analysis, we can define the specific conditions for EAD genesis, providing a more accurate theory of EADs. We use the nonlinear dynamical theory as a general framework to explain the roles of the major ionic currents and Ca cycling in EAD genesis, as detailed subsequently.

5.1 L-type Ca channel

$I_{\text{Cax}}$ has three dynamically important features: (i) the transient component reflecting normal voltage-dependent activation and inactivation kinetics; (ii) the window current component, resulting from the overlapping of the SSA and SSI curves; and (iii) the pedestal current due to incomplete inactivation at high voltages. Here we discuss how each of the components affect EAD genesis using the theoretical framework discussed earlier.

Window current. The window $I_{\text{Cax}}$ is known to play a very important role in EAD genesis. Our theoretical analysis reveals that its
role is to promote the QESs at the plateau voltages. Reducing the window $I_{\text{Ca,L}}$ by shifting the SSA or SSI curves can eliminate the QESs.\textsuperscript{33} Without the formation of the QESs, the voltage cannot remain in the plateau voltage range long enough to result in EADs. As shown in simulations,\textsuperscript{33} shifting the inactivation curve to reduce the window current eliminated EADs with little effect on voltage in the first few hundred milliseconds. This effect has been shown in our recent dynamic clamp experiments, revealing that minimal changes in the voltage dependence of activation or inactivation of $I_{\text{Ca,L}}$ can dramatically reduce the occurrence of EADs in cardiac myocytes exposed to different EAD-inducing conditions.\textsuperscript{39} Conversely, one can promote EADs by enhancing the window $I_{\text{Ca,L}}$ via shifting the SSA and SSI curves accordingly, a behaviour that has been shown by January et al.\textsuperscript{15} in an early computer simulation, and demonstrated in rabbit ventricular myocytes using the dynamic clamp technique.\textsuperscript{39} Note that the window current contributes to the formation of QESs in the window range and the slope of SSA curve determines the stability of the QES (namely the p-state). The instability of the QES leads to spontaneous re-activation of $I_{\text{Ca,L}}$ that facilitates the upstroke of an EAD.

Pedestal current. An increase in pedestal $I_{\text{Ca,L}}$ has a similar effect as an increase in window $I_{\text{Ca,L}}$, which is to promote QES formation. Differing from the window $I_{\text{Ca,L}}$, since the pedestal $I_{\text{Ca,L}}$ is present in and above the window range (Figure 7B), it can promote QESs in and above the window range. If the QES (p-state) is in the window range, it then can promote EADs. However, if the QES is above the window range, no EADs can occur even though APD can be very long since the QES is always stable. In addition, elevation of voltage due to pedestal $I_{\text{Ca,L}}$ speeds up the activation of $I_{\text{Kr}}$, which can shorten APD and suppress EADs under certain conditions, as shown in a recent study.\textsuperscript{33}

Activation and inactivation kinetics. Whereas the amplitudes of the window and pedestal $I_{\text{Ca,L}}$ play important roles in promoting the formation of QESs at the plateau voltage, $I_{\text{Ca,L}}$ activation and inactivation kinetics (the slopes of SSA and SSI curves and their time constants) play critical roles in the stability of the QES (p-state) and thus oscillations, as shown in the stability analysis.\textsuperscript{29} They also play an important role in regulating the transient component, which affects whether or not the system enters the basin of attraction of the oscillations. For example, speeding up the inactivation may cause stabilization of the p-state, resulting in repolarization failure, while slowing it can shorten APD and eliminate EADs due to missing the basin of attraction of oscillations (see Supplementary material online, Figure S4). Slowing inactivation to suppress EADs has also been shown in computer simulations using a detailed AP model in which slowing Ca-dependent inactivation suppressed EADs and resulted in a shorter APD.\textsuperscript{49}

These insights from the EAD theory may help us understand why the L-type Ca channel agonist BayK8644 promotes EADs,\textsuperscript{11,13,32} whereas overexpressing the mutant Ca-insensitive calmodulin CaM1234 to eliminate Ca-induced inactivation does not,\textsuperscript{24,32} although both increase $I_{\text{Ca,L}}$. BayK8644 increases conductance (thereby proportionately increasing the window current) and shifts the SSA and SSI curves to more negative voltages (by about $-10$ mV) without affecting their steepness. It also decreases both the Ca-dependent and voltage-dependent inactivation time constants.\textsuperscript{50,51} These changes all favour EAD genesis. CaM1234, on the other hand, reduces or eliminates Ca-dependent inactivation, reduces the slope of the inactivation of $I_{\text{Ca,L}}$, and increases the pedestal $I_{\text{Ca,L}}$.\textsuperscript{24} These changes tend to elevate the voltage out of the window range, suppressing EADs even though greatly prolonging APD.\textsuperscript{24,33}

5.2 Sodium channel

$I_{\text{Na}}$ is maximal during the AP upstroke, and almost completely inactivates during the AP plateau under normal conditions. However, under pathophysiological conditions, such as in type 3 long QT syndrome\textsuperscript{12,53} and heart failure,\textsuperscript{54} $I_{\text{Na}}$ may fail to inactivate completely, resulting in a small residual inward current during the repolarizing phase, called late $I_{\text{Na}}$, that promotes EADs.\textsuperscript{52,55,56} The role of the late $I_{\text{Na}}$ in EAD genesis is similar to the window or pedestal $I_{\text{Ca,L}}$, which promotes formation of the QESs in the $I_{\text{Ca,L}}$ window range (Figure 7B). Besides late $I_{\text{Na}}$, increased window $I_{\text{Na}}$ due to delayed inactivation\textsuperscript{53} or shifts in the voltage dependence of activation/inactivation may also promote EADs the same way as the window $I_{\text{Ca,L}}$. Theoretically, when $I_{\text{Kr}}$ is decreased and window $I_{\text{Na}}$ is increased, QESs can form in the voltage range of $I_{\text{Na}}$, activation window (between $-60$ mV and $-30$ mV) and cause spontaneous re-activation of $I_{\text{Na}}$ (and also $I_{\text{Ca,L}}$) to result in phase-3 EADs.

5.3 Potassium channels

Delayed rectifier $K$ currents ($I_{\text{K1}}$ and $I_{\text{Ks}}$). The delayed rectifier K current has both rapid ($I_{\text{K1}}$) and slow ($I_{\text{Ks}}$) components.\textsuperscript{57} Reduction or elimination of $I_{\text{K1}}$ and $I_{\text{Ks}}$ cause Type 1 and Type 2 long QT syndromes (LQT1 and LQT2),\textsuperscript{4,5} respectively. However, the roles of the two components in EAD genesis differ dramatically. Since $I_{\text{K1}}$ activates and inactivates rapidly,\textsuperscript{58} it reaches quasi-steady state quickly, which opposes steady-state inward currents to prevent the QES formation at the plateau voltage range. In other words, reducing $I_{\text{K1}}$ promotes EADs by allowing the QES to form. In addition, the fast kinetics of $I_{\text{K1}}$ may also play an important role in regulating the stability of the QES, which will require further analysis using a detailed $I_{\text{Kr}}$ model. $I_{\text{Kr}}$, on the other
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hand, plays a different role in EAD genesis. Since compared with other currents, its activation kinetics are much slower, exceeding 2 s at −20 mV, it acts as the slow variable which leads the system slowly across the oscillatory region to result in many cycles of oscillations. If is large, repolarization reserve is large and the QES in the plateau voltage disappear quickly, and thus the AP repolarizes normally. Due to the special role of in EAD genesis, if is absent, it may be difficult to induce EADs by blocking other outward currents (e.g. ) or increasing inward currents if there is no other slowly activating outward currents or slowly inactivating inward currents to lead the system through the oscillatory region in which EADs occur. As shown in our simulations using the LR1 model, if the activation time constant of is shortened to resemble , the range of the maximum conductance exhibiting EADs (and ultralong APDs) becomes very narrow, and repolarization failure occurs readily. However, is not the only slowly accumulating repolarization force controlling the duration of time spent in the window-voltage range required for EADs. For example, we recently showed that intracellular Na accumulation could play the same role by slowly increasing the outward Na–K pump current, leading to termination of EAD bursts. In cases in which is absent or does not contribute to repolarization, other slowly changing currents (e.g. the slowly inactivating late ) are required for facilitating voltage oscillations in the window range. However, for conditions of reduced repolarization reserve, only a small conductance is needed and thus may still be important even if it plays little role in repolarization under normal conditions.

Recognition of these different roles of and in EAD formation may provide mechanistic insights into the clinical outcomes of different LQT syndromes. In LQT2 and LQT3 patients, fatal cardiac events tend to occur during sleep or at rest (bradycardia-related), whereas in LQT1, events are often exercise-induced (tachycardia-related). Since is intact in LQT2 and LQT3 patients, slowing the heart rate allows the channels to enter more deeply closed states, delaying their activation kinetics and reducing repolarization reserve disproportionately during bradycardia. In LQT1, on the other hand, bradycardia does not preferentially reduce repolarization reserve since is already minimal. Instead, repolarization reserve is reduced during tachycardia, when adrenergic stimulation of is no longer counterbalanced by the adrenergic stimulation of . These rate-related differences are not absolute, however. In LQT1, is not completely absent, and bradycardia-related EADs can still occur, especially when isoproterenol is present.

It is well known that blocking or does not invariably cause EADs, even though APD can become substantially prolonged. The likely causes, based on the dynamics analysis are: (i) the QES (p-state) becomes stable by elevating the p-state out of the window-voltage range, so that no oscillations can occur; and (ii) the AP voltage remains too high in the early repolarizing phase to engage the basin of attraction of oscillations. The latter accounts for the observation that AP triangulation is more EAD-genic than is APD prolongation per se.

Transient outward K current (). As we showed recently, plays a non-intuitive role in EAD genesis, as illustrated in the examples shown in Figures 4 and 5. Both computer simulations and experiments show that with appropriate conductance and inactivation kinetics, this purely outward current can promote EADs. Since is a purely outward current, the finding that it can promote EADs is counterintuitive. However, from the nonlinear dynamics theory of EADs, the mechanism is readily apparent, as it becomes clear that the role of is to bring voltage to the range for oscillation (Figure 4), i.e. to bring the system into the basin of attraction for oscillations. We also show that this same mechanism is responsible for EADs induced by fibroblast–myocyte coupling, in which the coupling induces a transient outward current to the myocyte, similar to . Thus, in myocytes with little or no , such as rabbit at physiological heart rates, increasing up to a point tends to promote EADs, and beyond this point will suppress EADs. Conversely, in myocytes with very large native , such as...
atrial myocytes or mouse or rat ventricular myocytes, partially blocking \( I_{to} \) tends to promote EADs,\textsuperscript{38,67} whereas complete blocking \( I_{to} \) block may suppress them.\textsuperscript{18}

Inward rectifier K current (\( I_{K1} \)). The dual role of \( I_{K1} \) is to stabilize the resting membrane voltage during diastole and to provide a strong regenerative repolarizing force (confirmed by its negative slope region) during phase-3 of the AP. Due to its strong rectification, \( I_{K1} \) is small at the plateau voltage, and thus may have only a small effect on phase-2 EADs. However, reducing \( I_{K1} \) may promote phase-3 EADs. This can be understood as follows: reducing \( I_{K1} \) has little effect on the p-state, but may cause the s-state to shift to more negative voltages. Based on the bifurcation analysis (see Supplementary material online, Figure S3), the take-off potential of an EAD is bounded by the s-state, and thus moving the s-state towards more negative voltage can result in lower take-off potentials and larger amplitudes of EADs. Since the conductance of \( I_{K1} \) is regulated by extracellular \([K]o\), lowering \([K]o\) reduces \( I_{K1} \), which may be a major factor by which hypokalaemia promotes EADs.\textsuperscript{17,30,47}

5.4 Sodium–calcium exchange

As a predominantly inward current, increase in \( h_{NCX} \) tends to promote EADs,\textsuperscript{68} and, conversely, blocking \( h_{NCX} \) suppresses EADs.\textsuperscript{19,69,70} However, unlike \( i_{Ca,L} \), which reactivates regeneratively in the window voltage, \( h_{NCX} \) decreases with depolarization, and therefore cannot regeneratively enhance EAD amplitude unless intracellular Ca increases to maintain its driving force as membrane voltage depolarizes during the EAD upstroke. The main role of \( h_{NCX} \), therefore, is to favour the formation of QESs, specifically by shifting the s-state towards more negative voltages (similar to blocking \( I_{K1} \)), thereby promoting phase-3 EADs. Thus, \( h_{NCX} \) plays a similar role as the window and pedestal \( i_{Ca,L} \) and late \( I_{Na} \), to potentiate the formation of QESs. Without synergizing with the regenerative properties of \( i_{Ca,L} \) reactivation, however, \( h_{NCX} \) alone cannot produce EADs.

5.5 Intracellular Ca cycling

The association between intracellular Ca cycling and EADs was first shown in experiments by Priori and Corr.\textsuperscript{19} The roles of intracellular Ca cycling in EAD genesis are complex: (i) it promotes \( h_{NCX} \) to promote EADs as discussed in Section 5.4; (ii) in addition to \( h_{NCX} \), intracellular Ca directly regulates many other ionic currents (e.g. \( i_{Ca,L} \) and \( I_{Ks} \)), which affect repolarization reserve and thus the formation of the QESs; (iii) the Ca transient mediates Ca-dependent inactivation of \( i_{Ca,L} \) which affects the stability of the QES and the basin of attraction of the oscillations, as already discussed in Section 5.1; (iv) spontaneous SR Ca release and Ca waves can manifest as EADs\textsuperscript{21} through Ca-dependent ionic currents, mainly \( h_{NCX} \); (v) Ca signalling (e.g. via CaMKII) affects both Ca cycling and ionic currents influencing EAD genesis; (vi) bidirectional coupling of Ca and voltage can further potentiate or suppress EADs. For example, the presence of EADs due to increased \( i_{Ca,L} \) lengths APD, which causes extra Ca entry and more SR Ca release. Higher intracellular Ca increases \( h_{NCX} \), causing further membrane depolarization to potentiate EADs, or induces spontaneous Ca oscillations to manifest as EADs and DADs.\textsuperscript{19–21} However, the complex roles of Ca cycling on EADs cannot be fully understood until a rigorous nonlinear dynamics analysis (similar to the one for voltage present in this review) is, especially combining with computer simulations using spatially distributed detailed Ca cycling models which can simulate realistic intracellular Ca waves.

6. Conclusions

EADs are voltage oscillations occurring during the repolarization phase of the AP, resulting from nonlinear dynamical processes that are regulated by multiple factors (Figure 7A). In this article, we use nonlinear dynamics to provide a general theoretical framework which identifies the following requirements for EAD genesis:

(i) Properly balanced quasi-steady-state inward and outward currents that result in QES formation at the plateau voltage. This requirement is satisfied by reducing the conductance of outward currents and/or by increasing the conductance of inward currents (specifically, window and late \( I_{Na} \), window and pedestal \( i_{Ca,L} \), and \( h_{NCX} \)), which agrees with the concept of reduced repolarization reserve.\textsuperscript{12}

(ii) Hopf-homoclinic bifurcations in the fast subsystem that first generate and then terminate oscillations. This requirement is mainly determined by the kinetics of \( i_{Ca,L} \) and occurs in the voltage range where the SSA curve of \( i_{Ca,L} \) changes steeply. The steepness plays the key role in determining whether instabilities to develop so that \( i_{Ca,L} \) can reactivate spontaneously. This agrees with the observation that EADs occur in the \( i_{Ca,L} \) window-voltage range.\textsuperscript{16}

(iii) Properly balanced transient inward and outward currents during the early repolarization phase to ensure that voltage declines at the proper speed to bring the system into the basin of attraction of oscillations. This explains why tri sanitates promotes EADs.\textsuperscript{26,28}

All three conditions need to be completely satisfied for EADs to result. They are caused by the proper balance of transient and steady-state inward and outward currents with appropriate activation and inactivation kinetics, especially the kinetics of \( i_{Ca,L} \) and \( I_{Na} \). These three general requirements, as revealed by the dynamical theory, provide necessary and sufficient conditions for EAD formation.

Besides the mechanisms of EADs, what can we learn about therapeutics from this dynamics analysis? Since the three requirements need to be satisfied for EADs to occur, altering one or more of these required conditions in the appropriate manner should theoretically suppress EADs. However, an effective therapy must not impair normal excitation and excitation–contraction coupling. It is hard to alter the bifurcation point and basin of attraction in real systems, and thus targeting the QES formation may be the optimal choice. This is because, the QES formation requires the presence of one or more of the steady-state inward currents, namely, window and pedestal \( i_{Ca,L} \), window and late \( I_{Na} \) and \( h_{NCX} \). Since window and pedestal \( i_{Ca,L} \) window and late \( I_{Na} \) have little or small effects on AP and Ca cycling during a normal AP, but are crucial for EAD genesis, blocking these currents by shifting the corresponding SSI curves (Figure 7B) should be effective in suppressing EADs without adversely affecting normal excitation–contraction coupling. Both dynamic clamp studies focusing on \( i_{Ca,L} \) window modification\textsuperscript{39} and experimental results with the late \( I_{Na} \) blocker ranolazine\textsuperscript{55,56} support the feasibility of these strategies. Blocking \( h_{NCX} \) may be a less desirable strategy to prevent QES formation, since \( h_{NCX} \) plays such an important role in the excitation–contraction coupling. Likewise, targeting other currents, which can change one or more of the three dynamical factors to suppress EADs, seems less promising, since they may alter normal AP and excitation–contraction coupling.

Finally, whereas the dynamical theory of EADs outlined earlier addresses only the nonlinear dynamics of voltage in EAD genesis, the future incorporation of the dynamics of Ca cycling, and the novel dynamics due to voltage and Ca coupling, into a more complete dynamical theory may suggest other novel strategies for suppressing EADs and EAD-mediated arrhythmias.
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