How does β-adrenergic signalling affect the transitions from ventricular tachycardia to ventricular fibrillation?

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Received 16 July 2013; accepted after revision 27 November 2013

Aims

Ventricular tachycardia (VT) and fibrillation (VF) are the most lethal cardiac arrhythmias. The degeneration of VT into VF is associated with the breakup of a spiral wave of the action potential in cardiac tissue. β-Adrenergic (βAR) signalling potentiates the L-type Ca current (I_{CaL}) faster than the slow delayed rectifier potassium current (I_{Ks}), which transiently prolongs the action potential duration (APD) and promotes early after depolarizations. In this study, we aimed at investigating how βAR signalling affects the transition from VT to VF.

Methods and results

We used a physiologically detailed computer model of the rabbit ventricular myocyte in a two-dimensional tissue to determine how spiral waves respond to βAR activation following administration of isoproterenol. A simplified mathematical model was also used to investigate the underlying dynamics. We found that the spatiotemporal behaviour of spiral waves strongly depends on the kinetics of βAR activation. When βAR activation is rapid, a stable spiral wave turns into small fragments and its electrocardiogram reveals the transition from VT to VF. This is due to the transiently steepened APD restitution induced by the faster activation of I_{CaL} vs. I_{Ks} upon sudden βAR activation. The spiral wave may also disappear if its transient wavelength is too large to be supported by the tissue size upon sudden strong βAR activation that prolongs APD transiently. When βAR activation is gradual, a stable spiral wave remains such, because of more limited increase in both APD and slope of APD restitution due to more contemporaneous I_{CaL} and I_{Ks} activation.

Conclusion

Changes in APD restitution during βAR activation revealed a novel transient spiral wave dynamics; this spatiotemporal characteristic strongly depends on the protocol of isoproterenol application.

Keywords

β-Adrenergic stimulation • Ventricular tachycardia • Ventricular fibrillation • Spiral wave • Action potential duration restitution

Introduction

Sudden cardiac death is a major cause of death accounting for >300 000 deaths per year in the USA alone. Under normal conditions, cardiac action potentials originated from the sinoatrial node in a rate of ~75 b.p.m. periodically propagate in the tissue. When ventricular tachycardia (VT) is initiated, reentry of the propagation occurs and forms a spiral wave. This may stay as a stable spiral wave or degenerate into small fragments of waves, i.e. ventricular fibrillation (VF). These VT and VF events often terminate spontaneously.

In large mammalian hearts, action potential duration (APD) is determined by the delicate balance of outward and inward currents active during the plateau. The L-type Ca current (I_{CaL}) is the major inward current during this phase, thus I_{CaL} availability plays a key role in APD. The availability of I_{CaL} depends on the diastolic interval (DI) of the previous beat during which I_{CaL} recovers. Therefore, in most cases, APD increases with previous DI monotonically until a full recovery of I_{CaL}. This relation between the APD vs. its preceding DI is called APD restitution. The slope of the APD restitution curve governs the dynamical instability at both cellular and tissue levels. For example, a steep APD restitution is one of the major factors of both APD alternans and early after-depolarizations (EADs) at the single-cell level. At the tissue level, steep APD restitution is also a key factor for spatially discordant alternans by interacting with the
conduction velocity (CV) restitution, i.e. the relationship between CV and the preceding DI due to the availability of the sodium current (\(I_{Na}\)). Similarly, APD restitution together with CV restitution determines the dynamics of spiral waves as demonstrated in both experimental and theoretical studies.\(^{10–14}\)

The activation of \(\beta\)-adrenergic receptor (\(\beta\AR\)) by agonists such as isoproterenol (ISO) regulates many ionic currents and fluxes through the complex signalling pathways with differential timescales.\(^{15,16}\) We have shown the fact that \(\beta\AR\) activates \(I_{CaL}\) much quicker than the slow delayed rectifier potassium current (\(I_{Ks}\)) leads to a transient imbalance between inward and outward currents.\(^{15,17}\) This current imbalance can transiently prolong APD and potentiate EADs. The promotion of \(I_{CaL}\) steepens APD restitution while the increase of \(I_{Ks}\) flattens it. Thus, a quicker increase of \(I_{CaL}\) vs. \(I_{Ks}\) by ISO application can lead to a transiently steep APD restitution. In this study, we show how the activation of \(\beta\AR\) affects spiral wave dynamics in tissue by perturbing the APD restitution dynamically.

**Methods**

We use a physiologically detailed mathematical model of the rabbit ventricular myocyte as used in our previous investigations for spiral waves.\(^{18}\) Here, we combine this model with the \(\beta\AR\) branch of the Soltis and Saur-\(\alpha\)er\-men signalling pathway\(^{16}\) by introducing effects of protein kinase A (PKA) activation on \(I_{CaL}\), \(I_{Ks}\), troponin I, ryanodine receptor (RyR), phospholamban, and phospholemman. The activation kinetics of \(I_{Ks}\) by PKA is slowed down\(^{17}\) to match experimental observations.\(^{15}\) In this study, we investigate the transition from VT to VF during the administration of ISO. Spiral waves with the original model parameters are unstable and show a VF-like state. Thus, the maximum conductance of \(I_{CaL}\) (\(G_{Ca}\)) is decreased to 60% of the original value to stabilize the initial spiral wave before ISO application.

We also use a simplified mathematical model of a ventricular action potential to reveal the minimal dynamical mechanisms. We use a modified version of three-variable action potential model by Echebarria and Karma.\(^{19}\) It consists of membrane voltage, inactivation gate of \(I_{Na}\) and inactivation gate of \(I_{Ca}\). The maximum conductance of \(I_{Ca}\) and \(I_{Ks}\) are increased with an exponential function as shown by the experimental data

\[
G(t) = G_0 + G_{ISO}(1 - e^{-t/\tau})
\]

(1)

where \(G_0\) is \(G_{Ca}\), or \(G_{Ks}\) before ISO application, \(G_{ISO}\) is the increment by ISO, and \(\tau\) is the time constant of PKA-dependent target phosphorylation, which is 2.5 s for \(I_{CaL}\) and 20 s for \(I_{Ks}\). This difference in the activation timescales is within the experimental observation.\(^{15}\)

We assume that only the membrane voltage (\(V_m\)) diffuses in tissue through gap junctions. The governing equation is

\[
\frac{\partial V_m}{\partial t} = \frac{I}{C_m} + D_2 \left(\frac{\partial^2 V_m}{\partial x^2} + \frac{\partial^2 V_m}{\partial y^2}\right)
\]

(2)

where \(C_m = 1 \mu F/cm^2\) is the capacitance, \(I\) is the total transmembrane current, and \(D_2 = 0.0005 \text{ cm}^2/\text{ms}\) is the effective diffusion constant. We solve this equation using the operator splitting method\(^{20}\) for both the models. In the physiologically detailed model, we use Euler method with the variable time step of 0.01–0.1 ms to compute the action potential electrophysiology and Runge–Kutta method with a fixed time step of 0.01 ms for the \(\beta\AR\) signalling. A homogeneous tissue of 6 cm × 6 cm (400 × 400 grid points) is used for spiral waves. Space step (\(\Delta x\)) is 150 \(\mu\)m, which is similar to the length of the myocyte.

A stable spiral wave is induced by cross-field stimulation.\(^{21}\) All simulations start with the same condition. Then, we apply ISO with three different protocols: (i) gradual application (Figure 1A, left), (ii) sudden application of the same dose of ISO (Figure 1B, left), (iii) sudden application of a higher dose of ISO (Figure 1C, left).

The APD restitution curve is measured using the S1–S2 protocol.\(^{18}\) Pacing cycle length of is 300 ms for both the physiologically detailed model and the simplified model.

**Results**

Before ISO application, a stable spiral wave is initiated [voltage snapshot (1) in Figures 1A–C 0 s]. The corresponding electrocardiograms (ECGs) (of a 1.5 s period before ISO application) reveal VT-like fast activity (>600 b.p.m.) [bottom panels in Figures 1A–C (1)]. When ISO is applied gradually (with 10 steps, Figure 1A, left), both \(I_{CaL}\) and \(I_{Ks}\) are increased but the imbalance between them is limited. As the ISO administration proceeds, this leads to the slight meandering of the spiral wave towards the end of ISO administration [snapshot at time = 500 s, Figure 1A (1)]. However, the spiral wave does not breakup and its ECG remains a VT-like activity [Figure 1A (3), bottom].

When the same dose of ISO is applied suddenly (Figure 1B, left), \(I_{CaL}\) increases much quicker than \(I_{Ks}\) after ISO application (10–20 s), leading to a large current imbalance. The stable spiral wave becomes unstable and breaks up into small fragments when the difference between \(I_{CaL}\) and \(I_{Ks}\) becomes large [Figure 1B (2)]. During this period, the ECG resembles VF [Figure 1B (2), bottom]. At the end of ISO application, the activation of \(I_{Ks}\) matches that of \(I_{CaL}\) (Figure 1B, left). Spiral breakup turns back into spiral waves, which meander similarly as that in the gradual ISO application, revealing a VT-like ECG [Figure 1B (3)].

With sudden ISO application, the increase of dose will exacerbate the transient mismatch between \(I_{CaL}\) and \(I_{Ks}\) (Figure 1C; the total dose of ISO is increased from 20 to 500 nM). This leads to a transient larger prolongation of the action potential (Figure 2A) and the tissue size cannot support the spiral wave due to the large wavelength (wavelength = \(CV \times CV\)). After several rotations, the spiral wave disappears in 16.5 s [Figure 1C (3)]. This spontaneous termination of the spiral wave depends on the tissue size. If the tissue size is four times larger (e.g. 12 cm × 12 cm), so that it can support the transient large wavelength caused by the high dose of ISO administration, the spiral wave breaks up into fragments, similar to what is shown in Figure 1B (2) (results not shown).

The stability of the spiral wave has been linked to the steepness of the APD restitution and CV restitution.\(^{12,22}\) Since \(I_{Na}\) channel

\[
\frac{\partial V_m}{\partial t} = \frac{I}{C_m} + D_2 \left(\frac{\partial^2 V_m}{\partial x^2} + \frac{\partial^2 V_m}{\partial y^2}\right)
\]

(2)
availability is minimally influenced by βAR activation, the differential dynamical perturbation of APD restitution is likely to be key for the stability of spiral waves upon different protocols of ISO administration. We measure S1–S2 APD restitution during different protocols of ISO application (Figure 2B) and calculate their corresponding steepness (Figure 2C). Before the application of ISO (time 0 s), APD restitution is relatively flat (black). When ISO is applied suddenly, APD restitution becomes steep transiently (red, at time $t = 16$ s after ISO application), which corresponds to the spiral wave breakup (Figure 1B, snapshot at time $t = 16$ s). At the end of sudden application when both $I_{CaL}$ and $I_K$ are fully activated (time infinity), APD restitution becomes shallower (grey), which is
corresponding to the transition from spiral breakup to a stable spiral wave (Figure 1B, snapshot at time = 50 s). When ISO is applied gradually, however, the slope of the APD restitution curve is much shallower than that in the case of the sudden application (blue), which leads to a stable spiral wave (Figure 1A, snapshot at time = 500 s) but not spiral breakup.

To elucidate a minimal underlying mechanism of the spiral wave dynamics upon ISO application, i.e. APD restitution, we use a minimal model for the spiral wave dynamics. The corresponding timecourses of $G_{Ca}$ and $G_{Ks}$ are shown in Figure 3. As in the physiologically detailed model, sudden application of ISO initiates transient spatiotemporal chaos of action potential and VF-like ECG in the simplified model (Figure 3B). This transient VF failed to be induced by the gradual application of ISO (Figure 3A). We measure the maximum slope of the S1S2 APD restitution along ISO application (Figure 3C). This is always shallow when ISO is applied gradually but transiently steep when ISO is applied suddenly.

**Discussion**

By using both physiologically detailed model and minimal model, we investigate how βAR signalling affects spiral wave dynamics and thus modulates the transition between VT and VF. Our main findings are: (i) VT may degenerate into VF upon sudden application of ISO due to transiently steep APD restitution; (ii) VT remains such when ISO is applied gradually; (iii) sudden application of a high dose of ISO transiently prolongs APDs and results in spontaneous termination of VT/VF.

The slope of the APD restitution has been linked to spiral wave stability. Experimental and computational studies have shown that the spiral wave is destabilized when APD restitution becomes steep due to the head–tail interaction of the action potentials during reentry, which is similar to APD alternans. Although APD restitution is affected by many currents, $I_{CaL}$ plays a critical role since it is the major time-dependent inward current during the action potential plateau. Increasing $I_{CaL}$ steepens the APD restitution by enhancing its relative contribution to the APD restitution steepness. On the other hand, increasing $I_{Ks}$ flattens APD restitution by weakening its relative contribution. In fact, verapamil, the L-type Ca-channel blocker, turns VF into VT by flattening APD restitution. Experimental studies also have shown that ISO often steepens APD restitution and promotes arrhythmia.

The present study focuses on how different protocols of ISO application affect the transition between VT and VF by changing the APD restitution. When ISO is applied suddenly, it activates $I_{CaL}$ much faster than $I_{Ks}$. This steepens APD restitution transiently just after ISO application and then flattens it once $I_{Ks}$ is fully activated (Figure 2B). The transiently steep APD restitution causes spiral wave breakup and, accordingly, VT becomes VF at the beginning of ISO application. In addition to the slope of the APD restitution, APD can be transiently prolonged by the imbalance of inward and outward currents. In both experimental and computational studies, self-termination of spiral waves can occur when tissue size is small. When the amount of ISO is increased, the imbalance of inward and outward currents is also amplified. This APD prolongation due to the high dose of ISO promotes spontaneous termination of VT/VF.

![Figure 2](https://academic.oup.com/europace/article-abstract/16/3/452/612056)
As the critical signalling pathway that modulates excitation–contraction coupling, βAR can be activated in different manners. For example, the βAR surge tends to activate βAR quickly during acute physical or emotional activity\textsuperscript{30} and the spiral wave VT could trigger rapid βAR sympathetic response secondary to the reduced cardiac output. A sustained βAR response occurs during exercise, such that βAR activation state changes more gradually. These two different βAR kinetics were simulated by the sudden and gradual application of ISO. βAR stimulation activates $I_{CaL}$ much quicker than $I_{Ks}$, leading to a transiently much longer APD\textsuperscript{15,17} and steeper APD restitution (Figures 2A and B) at the initial stage of ISO application. Due to its dose dependence, a low ISO dose leads to a very limited mismatch of $I_{CaL}$ and $I_{Ks}$ (Figure 1A left). Therefore, in the gradual application, APD restitution is always shallow and the APD is not prolonged (Figure 2). On the other hand, as the same total dose of ISO is applied suddenly, a transiently large mismatch between $I_{CaL}$ and $I_{Ks}$ occurs. This steepens the APD restitution to exceed the stable limit of the spiral wave and causes transition from VT to VF at the early stage of ISO application. This transition reverses if the tissue size can support the transient large wavelength of spiral wave. Otherwise, VF terminates spontaneously.

In this study, the very rapid release of norepinephrine (NE) at sympathetic neuron varicosities upon activation is idealized as a step (instantaneous) change in [ISO]. Indeed, in conditions of emotional stress or light-or-flight response, NE released at the sympathetic nerve terminals is very rapid (like synaptic transmission), and [NE] is expected to rise rapidly in the cardiac interstitial space, given the extensive sympathetic innervation of the myocardium that brings sympathetic varicosities very close to the myocyte sarcolemma\textsuperscript{31} and the very brief synaptic delay ($\sim$0.5 to 3 ms\textsuperscript{23}). To examine the impact of [ISO] rising kinetics, we simulated an exponential increase in [ISO] (black in the inset of Figure S3, see Supplementary material online). Transition from VT to VF was induced for all time constants from $t_{[ISO]} = 0$ (step change) to $t_{[ISO]} = 1.8$ s (red area, see Supplementary material online, Figure S3). This agrees with experimental results, where cardiac sympathetic nerve stimulation causes rapid heart rate responses (within 1–2 s\textsuperscript{33}) or local infusion of ISO causes βAR myocyte effects within 1 or 2 beats\textsuperscript{34}.

βAR stimulation may also alter $I_{Na}$ by increasing peak current and slowing recovery from inactivation\textsuperscript{23}. The increase of peak $I_{Na}$ is not expected to affect spiral dynamics per se, although a larger tissue size might be required to support a spiral wave, because of increased CV. The relatively small slowing of $I_{Na}$ recovery steepens the CV restitution and could destabilize spiral waves. To test that we also included PKA-dependent $I_{Na}$ modulation, as described by Heijimen et al\textsuperscript{35} and simulated fast phosphorylation kinetics (like that of $I_{CaL}$, see Supplementary material online, Figures S1B and S2B) and slow kinetics (probably more realistic\textsuperscript{23,34} like that of $I_{Ks}$; see Supplementary material online, Figures S1C and S2C). During sudden ISO application, when $I_{Na}$ phosphorylation is slow, the spiral wave transition is the same as that without $I_{Na}$ phosphorylation (see Supplementary material online, Figure S1, C vs. A). When $I_{Na}$ phosphorylation is fast (see Supplementary material online, Figure S1B), transient wave-break still occurs (column b) but then disappears as peak $I_{Na}$ increases quickly (column d). The transient wave-break can be prolonged by increasing the tissue size (not shown). Upon gradual application of ISO, $I_{Na}$

\textbf{Figure 3} Effects of ISO application on spiral wave dynamics in a minimal model. (A) Spiral wave remains such upon gradual application of ISO. (B) Transient spiral wave breakup occurs upon sudden application of ISO. (C) APD restitution is steepened transiently by sudden application of ISO (red) but remains shallow upon gradual application of ISO (blue).
phosphorylation has no effect on the spiral wave transition regardless of its phosphorylation kinetics (see Supplementary material online, Figure S2, B and C vs. A).

Although PKA phosphorylation of I_{Cao} is controversial, βAR stimulation may increase peak I_{Cao} (~30%), much less than I_{Kr} and with a similar timecourse.36 Including I_{Kr} phosphorylation in our model predicts little effect on the transition of spiral dynamics (see Supplementary material online, Figures S1 and S2, D vs. A), as this is simply an additional component of the transient mismatch between overall repolarizing and depolarizing currents.

Intracellular Ca cycling is also expected to contribute to instability at both cellular and tissue level. The effect of βAR activation on Ca instability is also complex. On one hand, ISO increases the Ca influx by augmenting I_{Cao}, loading stores, and sensitizing RyRs, all of which tend to destabilize Ca cycling and be pro-arrhythmic.37 On the other hand, ISO increases the activities of SERCA pump and Na/K ATPase, which can stabilize calcium cycling.38 However, as we found previously,17 compared with the other βAR targets, the transient instability is dominated by the mismatch between the I_{Cao} and the I_{Kr} response kinetics. Therefore, we expect I_{Cao} and I_{Kr} to be dominant in the spiral wave dynamics studied here, where we focus on APD restitution effects on spiral dynamics during βAR activation. Although intracellular Ca may modulate spiral dynamics, it has little impact on the spiral wave dynamics in our model. Further investigations with a spatially distributed model of Ca dynamics are required to determine how intracellular Ca cycling is involved in ISO-induced wave instability.

Conclusion

In this paper, we describe a novel transient spiral wave dynamics during ISO application and show that spatiotemporal dynamics strongly depends on the application protocol. Understanding the mechanism of VT progression into VF and VT/VF termination may guide novel therapeutic strategies.

Supplementary material

Supplementary material is available at Europace online.

Conflict of interest: none declared.

Funding

This work was supported by University of California Davis Innovative Developmental Award (Y.X.) and National Institutes of Health grant R37-HL30077 and R01-HL105242 (D.M.B.) and K99-HL111334 (D.S.).

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