Contributions of Ion Channel Currents to Ventricular Action Potential Changes and Induction of Early Afterdepolarizations During Acute Hypoxia

Namit Gaur, Yoram Rudy, Livia Hool

Rationale: Variability in delivery of oxygen can lead to electric instability in the myocardium and the generation of arrhythmias. In addition ischemic heart disease and angina are associated with an increase in circulating catecholamines that further increases the risk of developing ventricular tachyarrhythmias.

Objective: We investigated the net effects of acute hypoxia and catecholamines on the cardiac action potential.

Methods and Results: We incorporated all published data on the effects of hypoxia on the late Na+ current (I_{Na-L}), the fast Na+ current (I_{Na}), the basal L-type Ca2+ channel current (I_{Ca-L}), and the slow (I_{Kr}) and rapid components of the delayed rectifier K+-current (I_{Ks}) in the absence and presence of β-adrenergic receptor (β-AR) stimulation into the Luo–Rudy model of the action potential. Hypoxia alone had little effect on the action potential configuration or action potential duration. However in the presence of β-AR stimulation, hypoxia caused a prolongation of the action potential and early afterdepolarizations (EADs) and spontaneous tachycardia were induced. Experiments performed in guinea pig ventricular myocytes confirmed the modeling results.

Conclusions: EADs occur predominantly because of the increased sensitivity of I_{Ca-L} to β-AR stimulation during hypoxia. β-AR stimulation is necessary to induce EADs as EADs are never observed during hypoxia in the absence of β-AR stimulation. (Circ Res. 2009;105:1196-1203.)

Key Words: hypoxia ■ adrenergic regulation ■ arrhythmia ■ ion channels ■ Ca2+ channels

Ventricular tachycardia and ventricular fibrillation are a major cause of death in patients with myocardial infarction and a reduced left ventricular ejection fraction. Typically arrhythmias occur as a result of re-entrant excitation or increased automaticity. Early afterdepolarizations (EADs) are depolarizations of the membrane potential that occur predominantly during phase 2 or 3 of the cardiac action potential and can degenerate to polymorphic ventricular tachycardia. EADs and triggered activity can induce reentrant arrhythmias. Generation of EADs requires an inward current that is large enough to depolarize the membrane potential.5

Variability in delivery of oxygen can lead to electric instability in the myocardium and the generation of arrhythmias. The cellular consequences of temporary acute hypoxia (seconds to minutes) differ significantly from chronic hypoxia (hours to days) or anoxia. A rapid decrease in oxygen supply to cardiac myocytes from 150 to 15 mm Hg is not energy limiting and does not deplete ATP but can alter the function of a number of cardiac ion channels. Under these conditions hypoxia increases late Na+ current (I_{Na-L}) while decreasing fast Na+ current (I_{Na}) in rat ventricular myocytes. It has been proposed that the increase in I_{Na-L} may be arrhythmogenic. In addition, acute hypoxia decreases the basal current through L-type Ca2+ channels (I_{Ca-L}) and the slow component of the delayed rectifier K+-channel (I_{Kr}) without affecting the rapid component (I_{Ks}). However, the net effects of acute hypoxia on action potential (AP) configuration in cardiac myocytes are not known.

Ischemic heart disease and angina are also associated with an increase in circulating and tissue catecholamines that increases the risk of developing ventricular tachyarrhythmias and sudden cardiac death. Hypoxia decreases the K_{Na} for activation of I_{Ca-L} by the β-adrenergic receptor (β-AR) agonist isoproterenol (Iso). However, hypoxia also increases the sensitivity of I_{Ks} to β-AR stimulation without altering I_{Kr}, and this could counteract the effects of hypoxia on I_{Ca-L}. In this study we used the Luo–Rudy model of a ventricular myocyte to determine the effects of acute hypoxia on the AP in the absence and presence of β-AR stimulation. By incorporating all published data on the effects of acute hypoxia (pO2 of 15 to 20 mm Hg) on Na+, Ca2+, and K+ currents, we find that in the absence of β-AR stimulation, hypoxia has little effect on the AP configuration and duration. However, in the presence of β-AR stimulation, hypoxia...
causes a prolongation of the AP and triggers EADs. We produce experimental data in guinea pig ventricular myocytes that support these theoretical findings and determine that EADs are generated predominantly because of hypoxia-induced increased sensitivity of \( I_{\text{Ca-L}} \) to \( \beta \)-AR activation.

**Methods**

**Cell Model**

The theoretical dynamic model of a mammalian ventricular AP, the Luo–Rudy model, provides the basis for the simulations.\(^{25}\) The model is predominantly based on guinea pig experimental data. The membrane ionic channel currents are formulated mathematically using Hodgkin–Huxley formalism. Ionic pumps and exchangers are also included in the model. The model accounts for processes that regulate intracellular ionic concentration changes of Na\(^+\), K\(^+\), and Ca\(^{2+}\). Intracellular processes represented in the model include Ca\(^{2+}\) uptake and Ca\(^{2+}\) release by the sarcoplasmic reticulum (SR) and the buffering of Ca\(^{2+}\) by calmodulin and troponin (in the myoplasm) and calsoasquerin (in the SR). For the Na\(^+\)/Ca\(^{2+}\) exchanger, the model uses a formulation based on conservation principle.\(^{24}\) Experimental data on voltage dependence of conductance and open time duration\(^{14,16}\) were used to formulate and include a model of \( I_{\text{Na-L}} \) in the model. \( \beta \)-AR effects were included in the model by using the \( K_{0.5} \) for enhancement of \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) caused by Iso as observed experimentally\(^{10,11}\) and by upregulation of SR Ca\(^{2+}\) uptake. Iso effect on inward rectifying potassium current \( (I_{\text{Ks}}) \) was also considered in simulations of the progressive effect of hypoxia on APs. Details of the model are provided in the Online Data Supplement at http://circres.ahajournals.org and the research section of http://rudylab.wustl.edu.

**Effects of Hypoxia on Ion Channels**

Hypoxia decreases \( I_{\text{Na}} \) and increases \( I_{\text{Na-L}} \) in ventricular myocytes.\(^{14,16}\) The effect of hypoxia on the sodium current was modeled by reducing conductance of \( I_{\text{Na}} \) by 10% and increasing the conductance of \( I_{\text{Na-L}} \) so that current was in the range of 0.1 to 0.5% of \( I_{\text{Na}} \) as seen experimentally.\(^{15}\) Hypoxia decreases basal \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) in the absence of \( \beta \)-AR stimulation.\(^{10,11}\) Hypoxia also decreases \( K_{0.5} \) for activation of \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) by Iso.\(^{10,11}\) Both of these effects were included in the model. These responses are reversible with an increase in oxygen tension to normoxia (room oxygen). During pacing, a stimulus of \(-80 \mu A/\mu F\) is applied for a duration of 0.5 ms. The model is paced with a conservative current stimulus carried by K\(^{+}\).\(^{26}\) A variable adaptive time step algorithm was used to simulate action potentials.\(^{23}\) Steady state was reached after 88 simulated beats. The ventricular action potential cell model was coded in C++ and run on Linux cluster nodes. For further details, see the Online Data Supplement.

**Isolation of Ventricular Myocytes and Patch-Clamp Studies**

Guinea pig ventricular myocytes were isolated as described previously.\(^{11}\) The current-clamp configuration of the patch-clamp technique was used to record action potentials in the myocytes (see the Online Data Supplement).

**Results**

**Effect of Hypoxia on \( I_{\text{Na}}, I_{\text{Na-L}}, I_{\text{Ca-L}}, I_{\text{Ks}}, I_{\text{Ks}} \) and the AP in the Absence of \( \beta \)-AR Stimulation**

We incorporated in the model the experimentally measured effects of hypoxia on \( I_{\text{Na}}, I_{\text{Na-L}}, I_{\text{Ca-L}}, I_{\text{Ks}}, \) and \( I_{\text{Ks}} \) in the absence of \( \beta \)-AR stimulation. In experiments, exposure to hypoxia reversibly reduces peak \( I_{\text{Ca-L}} \) by approximately 25% at 0 mV\(^{8,9,11,13,19,20}\) without shifting the current–voltage (I-V) relationship (Figure 1A).\(^{11,12}\) Figure 1B shows the simulated I-V relationship for \( I_{\text{Ca-L}} \) during control (normoxic) conditions and during hypoxia in the absence of Iso. There is a reduction in current amplitude but no shift in the I-V relationship, in good agreement with the experimental data. Acute hypoxia also causes a 2.19 ± 1.8% reversible decrease in steady state basal \( I_{\text{Ks}} \) current during a voltage-step to +50 mV (Figure 1C).\(^{10}\) Simulations demonstrate that hypoxia is associated with a 26.5% decrease in current at +50 mV, in good agreement with experimental data (Figure 1D).

We modeled the effects of hypoxia in the absence of \( \beta \)-AR stimulation on the AP at 2 pacing cycle lengths (CLs) of 300 and 1000 ms (Figure 2). As expected, the action potential duration (APD) decreases with increase in frequency of

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**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>AP</td>
<td>action potential</td>
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<tr>
<td>APD</td>
<td>action potential duration</td>
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<td>APP</td>
<td>action potential peak</td>
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<tr>
<td>( \beta )-AR</td>
<td>( \beta )-adrenergic receptor</td>
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<tr>
<td>Ca(^{2+})</td>
<td>intracellular Ca(^{2+}) concentration</td>
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<td>CL</td>
<td>cycle length</td>
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<tr>
<td>EAD</td>
<td>early afterdepolarization</td>
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<tr>
<td>( I_{\text{Ca-L}} )</td>
<td>L-type Ca(^{2+}) channel current</td>
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<tr>
<td>( I_{\text{Ks}} )</td>
<td>slow component of the delayed rectifier K(^+) current</td>
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<tr>
<td>( I_{\text{Na}} )</td>
<td>fast Na(^+) current</td>
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<tr>
<td>( I_{\text{NaCa}} )</td>
<td>Na(^+)/Ca(^{2+}) exchange current</td>
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<tr>
<td>( I_{\text{Na-L}} )</td>
<td>late Na(^+) current</td>
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<tr>
<td>Iso</td>
<td>isoproterenol</td>
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<tr>
<td>I-V</td>
<td>current–voltage</td>
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<tr>
<td>RMP</td>
<td>resting membrane potential</td>
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<td>SR</td>
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**Figure 1.** Hypoxia inhibits basal (no \( \beta \)-AR stimulation) \( I_{\text{Ca-L}} \) and basal \( I_{\text{Ks}} \). A and B, Experimental data (A) and simulated data (B) show inhibition of basal \( I_{\text{Ca-L}} \) by hypoxia. C and D, Experimental data (C) and modeled data (D) show inhibition of basal \( I_{\text{Ks}} \) by hypoxia.
stimulation, consistent with rate-dependent shortening of the APD. The APD90 for CL of 300 ms is 160 ms and the APD90 for CL of 1000 ms is 225 ms. When the effects of hypoxia on INa-L alone were incorporated in the model, the APD90 for CL of 300 ms is 162 ms and for CL of 1000 ms is 233 ms, with no change in resting membrane potential (RMP) or action potential peak (APP). Incorporating the effects of hypoxia on INa-L and INa in the model did not alter APD90 at CL of 300 ms (162 ms) or CL of 1000 ms (232 ms), and there was no change in RMP or APP. We conclude that neither INa-L nor INa alters the AP parameters during hypoxia. The effects of hypoxia on INa-L and INa were included in all the hypoxia simulations reported below.

When the effects of hypoxia on ICa-L, INa, and INa-L, but not on IKs, are incorporated in the model, the APD90 at CL of 300 ms is reduced by 4% to 153 ms and at CL of 1000 ms by 9% to 205 ms (Figure 2B). In some studies, a slight leftward shift in the voltage dependence of ICa-L during hypoxia has been reported.8,9 This shift was incorporated in the model but did not significantly alter the AP morphology although there was a slight decrease in APD (APD90 = 151 ms at CL of 300 ms and APD90 = 200 ms at CL of 1000 ms).

Next, we modeled the effect of hypoxia on INa, INa-L, and IKs but not on ICa-L (Figure 2C). Under these conditions, hypoxia causes a prolongation of APD90 by 8% to 173 ms at CL=300 ms and by 14% to 256 ms at CL=1000 ms. Finally, we modeled the known effects of hypoxia on all depolarizing and repolarizing currents (INa, INa-L, ICa-L, IKs, and IKr) (Figure 2D). There was very little effect on APD compared to normoxic controls (Figure 2A and Online Table I). To further examine a possible arrhythmogenic role for INa-L, we modeled the effect of hypoxia on INa-L at twice the value reported in published studies.14,16 There was no significant effect on APD or AP morphology (at CL of 1000 ms: APD90 = 234 ms, RMP = -88 mV, and APP = 45 mV). We conclude that hypoxia alone does not significantly alter the action potential.

Modeling β-AR Stimulation in the Absence and Presence of Hypoxia
The effects of β-AR stimulation on ICa-L are well documented.28 Binding of the β-AR leads to activation of cAMP and protein kinase A–dependent phosphorylation of the channel protein that then increases current magnitude and mode 2 open time. β-AR stimulation also leads to an increase in the magnitude of IKs as a result of direct phosphorylation of the channel.29 Neither the function of INa-L nor INa is regulated by protein kinase A or β-AR stimulation at concentrations less than 1 μmol/L.30 In the absence of hypoxia, 10 nmol/L Iso increases ICa-L to 72.1% of the current produced by a maximally stimulating concentration of Iso (1 μmol/L) in the same cell (Figure 3A). The peak current is increased 2.8-fold and is shifted 10.8 mV in the negative direction relative to the peak current recorded in control (no hypoxia).
Corresponding simulations in Figure 3B are in good agreement with the experimental data in Figure 3A. Exposure to 10 nmol/L Iso in the presence of hypoxia (Figure 3C) increases the magnitude of the peak current an additional 22.2% without further shifting the I-V relationship (−11.7 mV). Corresponding simulations in Figure 3D are in good agreement with the experimental data in Figure 3C.

In the presence of a saturating concentration of Iso (1 μmol/L), there is ~3-fold increase in steady-state \( I_{Ks} \) (Figure 3E).\textsuperscript{10} In the absence of hypoxia, 10 nmol/L Iso increases \( I_{Ks} \) to \( \sim 45.5\% \) of the current produced by 1 μmol/L Iso within the same cell (Figure 3E), while in the presence of hypoxia, 1 nmol/L Iso increases \( I_{Ks} \) \( \sim 56.5\% \) of the current produced by 1 μmol/L Iso in the same cell (Figure 3G).\textsuperscript{10} The magnitude of current density produced by 1 nmol/L Iso during hypoxia was comparable to the current density produced by 10 nmol/L Iso in room oxygen over the entire voltage range. Figure 3F demonstrates the effect of 10 nmol/L Iso on \( I_{Ks} \) during normoxia predicted by the model. Figure 3H demonstrates simulations for the effect of 1 nmol/L Iso on \( I_{Ks} \) in the presence of hypoxia.

We modeled the concentration dependence of \( I_{Ca,L} \) and \( I_{Ks} \) on Iso. In the absence of hypoxia, Iso increases \( I_{Ca,L} \) with a concentration that produces half-maximal activation (\( K_{0.5} \)) at 5.3 ± 0.7 nmol/L.\textsuperscript{11} When cells were exposed to hypoxia, the \( K_{0.5} \) for \( I_{Ca,L} \) was significantly decreased to 1.6 ± 0.1 nmol/L and the current was maximally stimulated with 10 nmol/L Iso.\textsuperscript{11} In the model, we used a \( K_{0.5} \) value of 5.3 nmol/L under normoxic conditions and 1.6 nmol/L Iso during hypoxia for enhancement of \( I_{Ca,L} \). Figure 4A shows that the model prediction of \( I_{Ca,L} \) enhancement by Iso is in good agreement with experimental data. In the absence of hypoxia, exposure of myocytes to 1 nmol/L Iso produces a subthreshold response for \( I_{Ks} \) and the current is maximally stimulated in the presence of 1 μmol/L Iso.\textsuperscript{10} The \( K_{0.5} \) for enhancement of the current in the absence of hypoxia is 18.3 ± 3.9 nmol/L. In the presence of hypoxia, 0.1 nmol/L Iso produced a threshold response and \( I_{Ks} \) was near-maximally activated at 10 nmol/L Iso. The \( K_{0.5} \) for enhancement of the current was significantly decreased to 1.88 ± 0.43 nmol/L under hypoxic conditions. Hypoxia did not alter the response to 1 μmol/L Iso, a maximally stimulating concentration of the agonist. In the model we used the \( K_{0.5} \) value of 15 nmol/L during normoxia (control) and a value of 1.5 nmol/L during hypoxia. These values are within the range of observed experimental values (Figure 4B).

**Effects of Hypoxia on \( I_{Ca,L} \) and the AP in the Presence of β-AR Stimulation**

The modeling results in Figures 1, 3, and 4 serve as validation that the model reproduces the experimental effects on individual membrane currents. In this section, we explore the effects of hypoxia and β-AR stimulation on the whole cell AP. Because hypoxia affects both the plateau-forming depolarizing current \( I_{Ca,L} \) and repolarizing current \( I_{Ks} \), in addition to affecting their sensitivity to Iso, it is unclear which of these currents plays a dominant role in shaping the AP morphology during hypoxia. Therefore, we first investigated the role of each channel in isolation and then combined their effects.

We investigated the effect of hypoxia on the AP while varying the amount of Iso to determine whether and at what Iso concentration arrhythmic disturbances occur. At low concentrations of Iso, we observed the generation of EADs. The threshold for EAD generation in the \( I_{Ca,L} \) hypoxic cell was 0.6 nmol/L at 1000-ms CL (Figure 5B and Online Table I). In the absence of Iso or at the same level of Iso (0.6 nmol/L), there was no evidence of rhythm disturbance in a control normoxic cell (Figure 5A and 5D; Online Table I). The APD\(_{90} \) in a control cell, with the addition of 0.6 nmol/L Iso, increased 8% to 244 ms at CL of 1000 ms and by 4% to 166 ms at CL of 300 ms with no changes in RMP or APP. When \( I_{Ca,L} \) was made hypoxic, the APD\(_{90} \) at CL 1000 ms was prolonged extensively by 82% (Online Table I) and EADs were observed (Figure 5B). At CL of 300 ms, APD\(_{90} \) was increased by 13% (relative to control) to 180 ms, but EADs were not generated.

At 1000-ms CL, prolongation of the AP led to the generation of an EAD. This is in contrast to the reduction of APD\(_{90} \) measured during hypoxia in the absence of Iso (Figure 2B). The concentration of Iso that induced EADs in the model (0.6 nmol/L) is close to \( K_{0.5} \) for activation of \( I_{Ca,L} \) by Iso during hypoxia (1.6 nmol/L) and to the threshold for activation of \( I_{Ks} \) under normoxic conditions (Figure 4). The prolongation of APD was not sufficient to generate EADs at 300 ms at any concentration of Iso.

**Effects of Hypoxia on \( I_{Ks} \) and the AP in the Presence of β-AR Stimulation**

Next, we investigated the effect of hypoxic \( I_{Ks} \) on the AP while varying the amount of Iso. We kept the values of other ion channels and transporters at normoxic levels in the model,
except \( I_{\text{Na-L}} \) and \( I_{\text{Na}} \), which were set at hypoxic values. We examined the effect of 0.6 nmol/L Iso in the presence of hypoxic \( I_{\text{Ks}} \) at CL of 1000 ms. From Figure 5E, it is seen that EADs were not observed for either CL of 1000 or 300 ms. The APD\(_{90}\) for CL of 1000 ms was increased by 26% compared to control (from 225 to 284 ms; Online Table I) and for CL of 300 ms 11% (from 160 to 177 ms). EADs were not generated over a wide range of Iso concentrations when only \( I_{\text{Ks}} \) channels were made hypoxic. This is attributable to the early enhancement of \( I_{\text{Ks}} \) as a result of increased sensitivity to Iso during hypoxia (\( K_{0.5}/H_{1.10} = 1.9 \text{ nmol/L} \)).

**Combined Effects of Hypoxia on \( I_{\text{Ks}} \) and \( I_{\text{Ca-L}} \) in the Presence of \( \beta\)-AR Stimulation**

Next, we modeled the combined effects of hypoxic \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) in the presence of \( \beta\)-AR stimulation. We progressively increased the concentration of Iso in the cell until EADs were observed at CL of 1000 ms. The level of Iso at which EADs first occurred was 0.5 nmol/L. The APD\(_{90}\) at CL of 300 ms was increased by 14% to 182 ms and for CL 1000 ms by 103% to 457 ms (Figure 5F; Online Table I). The concentration of Iso that induces EADs is lower than \( K_{0.5} \) for activation of \( I_{\text{Ks}} \) during hypoxia (1.9 nmol/L), implying that \( I_{\text{Ks}} \) current magnitude remains close to the basal hypoxic level and is not sufficient to counter the proarrhythmogenic effects of \( I_{\text{Ca-L}} \).

In summary, the simulations predict that during periodic pacing in the presence of \( \beta\)-AR stimulation, the primary reason for EAD generation during hypoxia is increased influx of calcium through \( I_{\text{Ca-L}} \). This effect may be augmented by the reduced outward current produced by \( I_{\text{Ks}} \) during hypoxia. However, the effect of hypoxia on \( I_{\text{Ks}} \) alone is insufficient to generate EADs. In addition, \( \beta\)-AR stimulation is necessary to induce EADs, because EADs are never observed under hypoxic conditions in the absence of \( \beta\)-AR stimulation.

**Effects of Hypoxia on Postpause APs**

During periodic pacing, the simulations did not generate EADs in a cell paced at CL of 300 ms. However, a case of clinical importance is periodic excitation followed by a pause.\(^{31} \) We examined the effect of a 1000-ms pause on a cell paced at a regular rate at 300-ms CL. In the absence of \( \beta\)-AR stimulation, EADs were not induced when the effects of hypoxia on \( I_{\text{Ca-L}} \) (Figure 6B), on \( I_{\text{Ks}} \) (Figure 6C), or the combined effects of hypoxia on \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) were included (Figure 6D).

The concentration of Iso that produced a threshold for EAD generation for hypoxic \( I_{\text{Ca-L}} \) was 0.6 nmol/L Iso (Figure 6B). At this concentration, EADs were not generated in a control or hypoxic \( I_{\text{Ks}} \) cell (Figure 6A and 6C). The combined effect of hypoxic \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) on a postpause AP is shown in Figure 6D. The threshold concentration of Iso for induction of EADs decreases from 0.6 to 0.4 nmol/L. We conclude that in a postpause AP, the primary cause for EAD formation is the effect of hypoxia on \( I_{\text{Ca-L}} \) in the presence of \( \beta\)-AR stimulation. Reduced \( I_{\text{Ks}} \) plays a secondary role and does not by itself induce EADs at Iso concentrations that generate EADs as a result of increased \( I_{\text{Ca-L}} \).

**Figure 5.** The effect of hypoxia and Iso on the AP and induction of EADs. A, Simulation data showing an AP paced at 300- and 1000-ms CL in a normoxic cell. B, Effect of hypoxic \( I_{\text{Ca-L}} \) and Iso. C, Effect of hypoxic \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) and no Iso. D, Effect of Iso in a normoxic cell. E, Effect of hypoxic \( I_{\text{Ks}} \) and Iso. F, Combined effect of hypoxic \( I_{\text{Ca-L}}, I_{\text{Ks}} \), and Iso.

**Figure 6.** The effect of a 1000-ms pause on induction of EADs (periodic pacing CL of 300 ms) in the absence and presence of Iso. Simulated AP pre- and postpause in: a control cell (normoxia) (A); cell with hypoxic \( I_{\text{Ca-L}} \) alone (B); cell with hypoxic \( I_{\text{Ks}} \) alone (C); and cell with combined hypoxic \( I_{\text{Ca-L}} \) and \( I_{\text{Ks}} \) (D).
Sustained Triggered Activity

In the model, when we only include the effect of Iso on inward rectifier K⁺ current (IKr),25 in the absence of hypoxia, there is no evidence of rhythm disturbance (data not shown). APs follow the periodic pacing and when pacing is stopped after 50 seconds, the cell becomes quiescent. Similar results were obtained for a hypoxic cell. However, in the presence of 1 nmol/L Iso with its time-dependent effect on Ikr, and instantaneous effect on IK, the hypoxic cell generates EADs. Shortly thereafter, the cell generates triggered non-paced beats (Figure 7). The pattern of EADs and triggered beats continues until pacing is stopped after the 50th beat. After cessation of pacing, the cell continues to beat periodically with a CL of 464 ms. There is no evidence of spontaneous activity in a control (nonhypoxic) cell in the presence of 1 nmol/L Iso (data not shown). We find that the spontaneous beats are generated by activation of ICa-L and not INa. The ICa-L during the spontaneous beat upstroke is substantially larger than ICa-L during the paced beat (Online Figure I).

Simulations show that the minimum diastolic cytosolic Ca²⁺ concentration (Caₐ) before the EADs occur is lower than the minimum diastolic Caₐ after EADs and/or sustained triggered activity. INaCa before the EADs occur is smaller than INaCa when EADs and/or spontaneous beats occur. When diastolic INaCa or Caₐ is clamped to values before EADs occur, the spontaneous beats are abolished (Online Figures II, III, and IV). Similarly, when extracellular Ca²⁺ is reduced from 1.8 mmol/L to 1.44 mmol/L in the simulations, both EADs and spontaneous beats are abolished. These results imply that Ca²⁺ plays an important role in the generation of EADs and spontaneous beats.

In the model, when we block the SR Ca²⁺ release channel to mimic the experimental protocol of ryanodine infusion, the sustained triggered activity persists. However, when we block ICa-L mimicking the action of Nifedipine, it is abolished (Online Figure V). These results imply that ICa-L is responsible for generation of the triggered activity and that spontaneous SR Ca²⁺ release is not required for its generation.

Experimental Effects of Hypoxia on the AP in the Absence and Presence of β-AR Stimulation

We investigated the model predictions of the effects of hypoxia in guinea pig ventricular myocytes. Reducing pO₂ from normoxic (pO₂ of 150 mm Hg) to hypoxic conditions (pO₂ of 17 mm Hg) for 60 seconds did not significantly alter RMP, APP, or APD (Figure 8A and Online Table I). We then exposed the myocyte to hypoxia in the presence of 1 nmol/L Iso, a concentration of the β-AR agonist that is subthreshold for activation of ICa-L and IK during normoxia (see Figure 4). The addition of Iso did not alter RMP (−82±3 versus −81±2 mV) or APP (45±2 versus 44±2 mV) but significantly increased APD by 11% (182±20 versus 201±25 ms, P<0.05, n=5). Increasing the concentration to 3 nmol/L Iso significantly increased APD by 37% without altering RMP or APP (Figure 8B and Online Table I). Application of dantrolene to block ryanodine receptor release of Ca²⁺ did not prevent the prolongation of AP (see Online Figure VIII). Three of 7 cells exposed to hypoxia +3 nmol/L Iso generated EADs and then started to beat spontaneously at CL of ~500 ms, close to the CL of 464 ms predicted by the model (Figure 8C and 8D). The upstroke velocity (dV/dt)max of a paced beat was 138 mV/ms and of a spontaneous beat 73.5 mV/ms (Online Table I). This confirms the model prediction that the upstroke in a paced beat is generated by INa and in a spontaneous beat is by ICa-L. Importantly, 3 nmol/L Iso did not alter AP parameters under normoxic conditions (RMP=−78±3 versus −79±4 mV, APP=41±5 mV versus 38±6 m, APD=238±55 versus 233±58 ms, all P>0.05, n=3), indicating that it is the combination of acute hypoxia and β-AR stimulation that is responsible for AP prolongation and EAD formation.

Discussion

Although it is well recognized that arrhythmias are a significant cause of death in ischemic heart disease, the role of acute hypoxia in induction of arrhythmia is not well understood. We incorporated all published data reporting the
effects of acute hypoxia on $I_{Na}$, $I_{Na-L}$, $I_{Ca-L}$, $I_{Ks}$, and $I_{Kr}$ into the Luo–Rudy model of a cardiac ventricular AP and determined the effect on AP morphology and APD. In the absence of β-AR stimulation, hypoxia has little effect on the AP (Figures 2, 5, and 6) even when we modeled the effects of hypoxia on $I_{Na-L}$ and $I_{Na}$ at twice the rates reported in published studies.11–16 In the presence of β-AR stimulation in a paced cell, EADs are generated only at CL of 1000 ms when the effects of hypoxia on $I_{Ca-L}$ are modeled alone or together with hypoxic $I_{Ks}$ (Figure 5). Experimental results confirm the modeling results (Figure 8, Online Figure VIII, and Online Table 1). Similar results were obtained for pause-induced EADs at a shorter CL of 300 ms (Figure 6). EADs quickly degenerate into spontaneous tachycardia only in a hypoxic cell in the presence of 1 nM/L iso when we also include the effect of iso on $I_{K1}$ (Figure 7).

Oxygen is the substrate for the production of reactive oxygen species. A rapid decrease in oxygen tension that is not energy limiting (and not ATP depleting; thus, ATP-dependent potassium current [$I_{KATP}$] is not activated) is associated with a decrease in cellular reactive oxygen species and a more reduced cellular redox state.11,13,32–35 Electrophysiological effects of acute myocardial ischemia where there is complete cessation of perfusion and $I_{KATP}$ plays an important role have been investigated elsewhere.36 The increase in sensitivity of $I_{Ca-L}$ to iso during hypoxia occurs as a result of modification of thiol groups on the channel or a regulatory protein such as protein kinase A because exposing myocytes to dithiothreitol could not induce EADs in a native cell during hypoxia or when modeling hypoxia alone. However, in the presence of β-AR stimulation, an increase in calcium influx through $I_{Ca-L}$ prolongs APD and triggers EADs. The frequency of EADs is influenced by the modal gating of $I_{Ca-L}$. Increased ratios of channels gating in mode 2 (that occurs with β-AR stimulation) are associated with increased frequency of EADs.37

Sympathetic stimulation increases the risk of arrhythmia. Beta-blockers are the only class of antiarrhythmics that have been demonstrated to decrease mortality.3 The results of this study are consistent with previously published data indicating that decreasing calcium influx through the channel or decreasing adrenergic stimulation can reduce the incidence of EADs. Ca$^{2+}$/calmodulin–dependent protein kinase II inhibitory peptide can eliminate EADs and ventricular tachycardia,38 as can protein kinase A inhibitors and β-AR antagonists.21,39–41 We conclude that $I_{Ca-L}$ is the primary initiator of EADs and spontaneous tachycardia occurs during hypoxia as a result of increased sensitivity of the channel to β-AR stimulation.

Sources of Funding

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Disclosures

None.

References


Contributions of Ion Channel Currents to Ventricular Action Potential Changes and Induction of Early Afterdepolarizations During Acute Hypoxia
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ONLINE DATA SUPPLEMENT

CONTRIBUTIONS OF ION-CHANNEL CURRENTS TO VENTRICULAR ACTION POTENTIAL CHANGES AND INDUCTION OF EARLY AFTERDEPOLARIZATIONS DURING ACUTE HYPOXIA

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EXPANDED MATERIALS AND METHODS

Equations for Theoretical Modeling

For a complete list of model equations and definitions of the Luo-Rudy Model please see the research section of Rudy Lab website: http://rudylab.wustl.edu.

Below are the equations that are added and/or modified for the simulations in this article.

Definitions of Variables and Parameters

$K50_{Iso}$: $K_{0.5}$ of activation of L-type Ca channel current ($I_{Ca-L}$) or slowly activated delayed rectifier potassium current ($I_{Ks}$) in response to Isoproterenol (Iso) in nmol/L

$Isofactor$: change factor of $I_{Ca-L}$ or $I_{Ks}$ due to Iso

$Iso$: concentration of Iso (nmol/L)

$ilca$: calcium ($Ca^{2+}$) current through L-type channels ($\mu A/\mu F$)

$ilcana$: sodium ($Na^{+}$) current through L-type channels ($\mu A/\mu F$)

$ilcak$: potassium ($K^{+}$) current through L-type channels ($\mu A/\mu F$)

$ilcatot$: total current through L-type channels ($\mu A/\mu F$)

$d$: voltage dependent activation gate of $I_{Ca-L}$

$f$: voltage dependent inactivation gate of $I_{Ca-L}$

$fca$: $Ca^{2+}$ dependent inactivation gate of $I_{Ca-L}$

$ibarca$: max. $Ca^{2+}$ current through L-type channels ($\mu A/\mu F$)

$ibarna$: max. $Na^{+}$ current through L-type channels ($\mu A/\mu F$)

$ibark$: max. $K^{+}$ current through L-type channels ($\mu A/\mu F$)

$iks$: current through slowly activated delayed rectifier $K^{+}$ channels ($\mu A/\mu F$)
\(gks\): max. conductance of \(I_{Ks}\) (mS.µF\(^{-1}\))

\(xs1\): fast activation gate of \(I_{Ks}\)

\(xs2\): slow activation gate of \(I_{Ks}\)

\(eks\): reversal potential of \(I_{Ks}\) (mV)

\(ainap\): rate of activation of late Na\(^+\) current (\(I_{Na-L}\)) (ms\(^{-1}\))

\(binap\): rate of deactivation of \(I_{Na-L}\) (ms\(^{-1}\))

\(p\): activation gate of \(I_{Na-L}\)

\(pss\): steady state value of activation gate of \(I_{Na-L}\)

\(taup\): time constant of activation gate of \(I_{Na-L}\)

\(gna\): max. conductance of fast Na\(^+\) current (mS.µF\(^{-1}\))

\(inap\): late Na\(^+\) current (µA.µF\(^{-1}\))

\(ina\): fast Na\(^+\) current (µA.µF\(^{-1}\))

\(v\): membrane potential (mV)

\(ena\): reversal potential of Na\(^+\) current (mV)

\(iup\): rate of Ca\(^{2+}\) uptake from myoplasm to network sarcoplasmic reticulum (NSR) (mmol.L\(^{-1}\).ms\(^{-1}\))

\(iupbar\): max. rate of Ca\(^{2+}\) uptake from myoplasm to NSR (mmol.L\(^{-1}\).ms\(^{-1}\))

\(cai\): myoplasmic Ca\(^{2+}\) concentration (µmol.L\(^{-1}\).µF\(^{-1}\))

\(kmup\): half saturation concentration of \(iup\) (mmol.L\(^{-1}\))

\(iki\): current through inward rectifier K\(^+\) channels (\(I_{K1}\)) (µA.µF\(^{-1}\))

\(gki\): max. conductance of \(iki\) (µS.µF\(^{-1}\))

\(eki\): reversal potential of K\(^+\) currents (mV)

\(kin\): inactivation gate of \(I_{K1}\)
Model Equations

L-Type Calcium Current, $I_{Ca-L}$

$K50Iso = 5.3$ for Control Conditions

$K50Iso = 1.6$ for Hypoxic Conditions

$ Isofactor = 1 + \frac{3}{1 + 10^{\log_{10}(K50Iso) - \log_{10}(Io)}} \text{ for Control Conditions} $

$ Isofactor = 0.75 + \frac{3}{1 + 10^{\log_{10}(K50Iso) - \log_{10}(Io)}} \text{ for Hypoxic Conditions} $

$ ilca = Isofactor*d*f*ica*ibarca $

$ ilcana = Isofactor*d*f*ican*ibarna $

$ ilcak = Isofactor*d*f*ica*ibark $

$ ilcatot = ilca + ilcana + ilcak $

$ Iso = 0.0001 (~0) \text{ for non } \beta\text{-adrenergic conditions} $

Slowly Activating Delayed Rectifier Potassium Current, $I_{Ks}$

$K50Iso = 15$ for Control Conditions

$K50Iso = 1.5$ for Hypoxic Conditions

$ Isofactor = 0.3 + \frac{0.6}{1 + 10^{\log_{10}(K50Iso) - \log_{10}(Io)}} \text{ for Control Conditions} $

$ Isofactor = 0.22 + \frac{0.6}{1 + 10^{\log_{10}(K50Iso) - \log_{10}(Io)}} \text{ for Hypoxic Conditions} $

$ iks = Isofactor*gks*x1*x2*(v-eks) $

$ Iso = 0.0001 (~0) \text{ for non } \beta\text{-adrenergic conditions} $

Late Sodium Current, $I_{NaL}$

$ ainap = 19*exp\left(\frac{v}{16.5}\right) $ 

$ binap = 0.2*exp\left(\frac{-v}{20}\right) $
\[ pss = \left( \frac{ainap}{ainap + binap} \right) \]

\[ taup = \left( \frac{1}{ainap + binap} \right) \]

\[ p = pss - (pss - p) \cdot \exp \left( -\frac{dt}{taup} \right) \]

\[ inap = 0.00007 \cdot gna \cdot p^3 \cdot (v - ena) \] for Control Conditions

\[ inap = 0.00018 \cdot gna \cdot p^3 \cdot (v - ena) \] for Hypoxic Conditions

**Fast Sodium Current, \( I_{Na} \)**

\[ gna = 16 \] for Control Conditions

\[ gna = 14.4 \] for Hypoxic Conditions

**Transient Outward \( K^+ \) current, \( I_{to1} \)**

A recently published model of \( I_{to1} \)^1 was used in the simulations when looking into the effects of Hypoxia and Iso in the presence of \( I_{to1} \).

**SR \( Ca^{2+} \) Uptake**

\[ iup = 1.5 \cdot iupbar \cdot \left( \frac{cai}{cai + kmup} \right) \] for \( \beta \)-adrenergic conditions

\[ iup = iupbar \cdot \left( \frac{cai}{cai + kmup} \right) \] for non \( \beta \)-adrenergic conditions

**Effect of Iso on \( I_{K1} \)**

Experiments by Koumi et al.\(^2\) indicates that the open probability of \( I_{K1} \) channels decreases to 21±4\% of its normal value during exposure to Iso.

We incorporate this effect in the model by reducing \( I_{K1} \) to 25\% of its normal value.

\[ iki = 0.25 \cdot gki \cdot kin \cdot (v - eki) \]

**Time-dependent changes of \( I_{Ca-L} \) and \( I_{Ks} \) due to Iso**
For less than 50 beats

\[ Isofactor = Isofactor \times (i/50) \], where \( i \) is the beat number

For more than 50 beats

\[ Isofactor = Isofactor \]

**Effect of Nifedipine**

Effect of Nifedipine is simulated as complete block of \( I_{Ca-L} \).

**Effect of Nisoldipine**

Effect of Nisoldipine is simulated as 30% block of \( I_{Ca-L} \).

**Effect of Ryanodine**

In simulations, effect of ryanodine is taken as complete block of SR Ca release.

**Ca stores and buffers**

In the model, the internal Ca store sarcoplasmic reticulum (SR) is divided into two compartments: network SR (NSR) and junctional SR (JSR). Ca is buffered in the JSR by the low-affinity Ca buffer calsequestrin (CSQN) and this ensures that there is a sufficient pool of releasable Ca from the junction. Ca in the myoplasm is buffered by troponin (TRPN) and calmodulin (CMDN).

**Ca cycling**

During an action potential (AP), the dominant trigger for SR Ca release is the rate of rise of Ca in myoplasm due to \( I_{Ca-L} \) (contributions from all other currents carrying Ca, e.g. \( I_{NaCa} \), are also included in the model). The release occurs with rates that ensure sufficient depletion of Ca stores and robust termination of release within a few milliseconds after its initiation. The release causes a peak transient of \( [Ca_i] = 1 \mu mol/L \) under normal conditions. The released Ca reenters the NSR through an uptake mechanism. It is then transferred to the
JSR through a translocation process. Ca release can also occur through an internal process. If at any point during an AP Ca in the JSR exceeds a certain threshold, Ca release from JSR can occur. This process simulates spontaneous overload induced calcium release (SOICR). Ca ions that remain in the myoplasm are extruded from the cell by $I_{NaCa}$. For details, we refer readers to references\textsuperscript{3-5} and to http://rudylab.wustl.edu.

**Limitations of the Ca cycling model**

In the model we do not explicitly take into consideration the stochastic and discrete nature of subcellular Ca release processes at the molecular scale. Using a detailed and distributed molecular model of Ca cycling is beyond the scope of this paper. However, the macroscopic (global) model used in the simulations accounts for all processes that are investigated in this study. The results demonstrate that Ca overload occurs when EADs occur and that Ca overload plays a role in the genesis and sustenance of triggered activity.

**Isolation of ventricular myocytes and patch-clamp studies**

For all studies involving myocytes adult Tricolor guinea-pigs (*cavea porcellis*) weighing between 200 and 250g were used. A total number of 26 guinea-pigs were anesthetized with intraperitoneal injection of pentobarbitone sodium (240 mg/kg) prior to excision of the heart as approved by The Animal Ethics Committee of The University of Western Australia in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NH&MRC, 7\textsuperscript{th} Edition, 2004). Guinea-pig ventricular myocytes were isolated as described previously.\textsuperscript{6} The current-clamp configuration of the patch-clamp technique was used to record action potentials in the myocytes.
External solution contained (in mmol/L): 140 NaCl, 5.4 KCl, 2.5 CaCl$_2$, 0.5 MgCl$_2$, 5.5 Hepes, 11.0 glucose, pH 7.4 at 37°C. Upon achieving whole cell patch configuration, action potentials were elicited by applying a 0.4 ms pulse of 60-100 nA using a basic cycle length of 1000 ms. Microelectrodes were made from borosilicate glass (Sigma) using a microelectrode puller (PC-10, Narishige, Tokyo, Japan) and back-filled with solution containing (in mmol/L): 120 potassium-L-glutamate, 20 KCl, 10 NaCl, 2 MgCl$_2$, 5 Hepes, 0.1 EGTA, 5.0 MgATP, pH 7.05 at 37°C. A total of 30 μmol/L CaCl$_2$ was added giving a free Ca$^{2+}$ concentration of 115 nmol/L as calculated using the MAXchelator program (http://www.standford.edu/~cpatton/webmaxc/webmaxclite115.htm). Membrane responses were filtered at 5 kHz, digitized at 20 kHz and analyzed using an Axopatch 200B amplifier in conjunction with pCLAMP 10.2 acquisition and analysis software (Molecular Devices).

Solutions were made hypoxic by bubbling the reservoir leading to the bath with 100% nitrogen and using a combination of stainless steel (Alltech) and silastic tubing (Cole-Parmer) for the delivery of solutions. Within 1-2 min, a consistent PO$_2$ of 17 mmHg was achieved assessed using an oxygen sensitive probe (Precision Measurement Engineering) as described previously$^6$. When cells were placed in the chamber, a fast-flow system was used to rapidly (< 1 sec) change the extracellular solutions bathing the myocyte from which action potentials were being recorded, and minimize contamination of the cell from extracellular solutions containing a higher PO$_2$. All hypoxia experiments were performed at a PO$_2$ of 17 mmHg consistent with previously published experimental data we incorporated into the model. To
minimize the possibility of oxidative degradation, ascorbic acid is commonly added to solutions containing the β-AR agonist isoproterenol (Iso). However, to prevent any direct effect of the antioxidant on channel function, ascorbic acid was omitted from all solutions and solutions containing Iso were prepared fresh and changed every 60 min.

**Measurement of Ca$^{2+}$ transients in ventricular myocytes**

Changes in intracellular calcium were recorded using the fluorescent indicator Fura-2 AM (1 μmol/L, Molecular Probes) while cells were patch-clamped. Internal electrode solution and extracellular solution were both supplemented with 1 μmol/L Fura-2AM. Fluorescent ratios at 340/380 nm ex, 510nm em were measured using stream acquisition at 23 ms intervals on a Hamamatsu Orca ER digital camera attached to an inverted Nikon TE2000-U microscope. Metamorph 6.3 was used to quantify the signal by manually tracing myocytes. An equivalent region not containing cells was used for background and was subtracted. Ratiometric 340/380 nm fluorescence was plotted relative to baseline fluorescence acquired prior to cell stimulation and was assigned a value of 1.0. All experiments were performed at 37°C.
ADDITIONAL SUPPORTING RESULTS

Role for KChIP2

The K+ channel interacting protein 2 (KChIP2) is an auxiliary subunit for Kv4.2 and has been reported to modulate a number of cardiac ion channel currents. We incorporated KChIP2 as transient outward K+ current (Ito1) in our simulations (g = 0.2 mS/cm²) and examined the effect on the AP. In the presence of hypoxia alone, Ito1 had little effect on AP configuration and AP duration. However in the presence of hypoxia and Iso, Ito1 decreased the threshold for generation of EADs by 28% (Online Figure VIA). When the conductance for Ito1 was included in simulations as g = 0.4975 mS/cm² the threshold for generation of EADs in presence of Iso was decreased by 35%. When nisoldipine (an I_{Ca-L} blocker) was included in the simulations, the EADs were eliminated (Online Figure VIB). We conclude that Ito1 decreases the threshold for induction of EADs through its indirect effect (via the transmembrane potential) to increase I_{Ca-L}.

Effect of hypoxia and Iso on intracellular calcium

We measured calcium transients in 4 ventricular myocytes loaded with Fura 2 during AP stimulations. In the presence of hypoxia calcium transients were similar to transients recorded in control (normoxic) conditions (inset middle Online Figure VIIA and VIIB). In the presence of 3 nmol/L Iso and hypoxia, the calcium transient was significantly larger (inset middle Online Figure VIIIC). We modelled the effect of hypoxia in the absence and presence of 0.5 nmol/L Iso on changes in intracellular Ca^{2+} and free junctional SR Ca^{2+} concentration
(Online Figure VII); results demonstrate that the modelled data are in good agreement with the experimental data.

**Effect of dantrolene on AP configuration in ventricular myocytes**

We examined whether release of calcium from the SR altered the AP configuration during hypoxia. Ventricular myocytes were stimulated and APs recorded in external solution containing 20 μmol/L dantrolene, an inhibitor of calcium release from RyRs in the SR. In 6 cells, dantrolene did not affect AP configuration in control (normoxic) solution (Online Figure VIII A) or during hypoxia (Online Figure VIII B). Similar to effects recorded in the absence of dantrolene (Figure 8), 3 nmol/L Iso caused a prolongation of the AP duration in the presence of hypoxia and induced EADs (Online Figure VIII C). We conclude that calcium release from the SR does not contribute to the prolongation of AP and development of EADs during hypoxia and Iso.
ONLINE DATA SUPPLEMENT FIGURE LEGENDS

Online Figure I. Ionic current underlying upstroke of paced AP and spontaneous AP. (A) Spontaneous AP (B) Paced AP (C) Upstroke in spontaneous AP is caused by $I_{\text{Ca-L}}$ (D) Upstroke in paced AP is caused by $I_{\text{Na}}$. Note different $I_{\text{Na}}$ and $I_{\text{Ca-L}}$ scales.

Online Figure II. Cytosolic Ca concentration ($Ca_i$) and Na$^+$-Ca$^{2+}$ exchange current ($I_{\text{NaCa}}$) in a hypoxic + 1 nmol/L Iso cell. (A) Phase of AP progression when EADs degenerate into spontaneous beats (during pacing) (B) Phase of sustained triggered (spontaneous) activity after cessation of pacing (C) $Ca_i$ in (A) (D) $Ca_i$ in (B) (E) $I_{\text{NaCa}}$ in (A) (F) $I_{\text{NaCa}}$ in (B)

Online Figure III. Abolition of a spontaneous beat due to $Ca_i$ clamp. (A) Abolition of spontaneous beat in phase of AP progression when EADs degenerate into spontaneous beats (during pacing) (B) Abolition of spontaneous beat in the phase of sustained triggered (spontaneous) activity after cessation of pacing (C) $Ca_i$ in (A). (D) $Ca_i$ in (B). (E) $I_{\text{NaCa}}$ in (A). (F) $I_{\text{NaCa}}$ in (B).

Online Figure IV. Abolition of spontaneous beat due to $I_{\text{NaCa}}$ clamp. (A) Abolition of spontaneous beat in phase of AP progression when EADs degenerate into spontaneous beats (during pacing) (B) Abolition of spontaneous beat in the phase of sustained triggered (spontaneous) activity beats after cessation of pacing (C) $Ca_i$ in (A) (D) $Ca_i$ in (B) (E) $I_{\text{NaCa}}$ in (A) (F) $I_{\text{NaCa}}$ in (B)
**Online Figure V.** Effect of block of ryanodine receptors (RyR) and $I_{\text{Ca-L}}$ on development of spontaneous beats. (A) Control AP (B) AP during RyR block (C) Membrane voltage during $I_{\text{Ca-L}}$ block (D) $I_{\text{Ca-L}}$ in absence of RyR and $I_{\text{Ca-L}}$ block (E) $I_{\text{Ca-L}}$ during RyR block (Ryanodine+) which does not abolish spontaneous beats (F) $I_{\text{Ca-L}}$ during application of L-type channel blocker, Nifedipine (Nifedipine+) which abolishes spontaneous beats. Absence of RyR block is denoted as Ryanodine-. Absence of $I_{\text{Ca-L}}$ block is denoted as Nifedipine-.

**Online Figure VI.** Effect of Hypoxia and Iso in the presence of $I_{\text{lo1}}$. (A) EADs are observed in the presence of hypoxia and 1nmol/L Iso. (B) Adding nisoldipine eliminates these EADs. Nisoldipine is simulated as 30% block of $I_{\text{Ca-L}}$.

**Online Figure VII.** Simulated and experimental effects of hypoxia and Iso on intracellular calcium. Simulated AP, free cytosolic Ca and free junctional SR Ca concentration during (A) Control (B) Hypoxia (C) Hypoxia in the presence of EAD-inducing Iso concentration (0.5 nmol/L). Experimentally measured changes in intracellular calcium, recorded during APs in a ventricular myocyte, are shown in insets labeled “Experiment” for (A) control (normoxic) extracellular solution, (B) during hypoxia and (C) during hypoxia + 3 nmol/L Iso.
**Online Figure VIII.** Effect of dantrolene on APs in a ventricular myocyte.
Dantrolene inhibits SR calcium release through RyRs. A) Control APs in the absence and presence of dantrolene (B) APs in the same cell in the presence of dantrolene and hypoxia (C) APs in the same cell in the presence of dantrolene, hypoxia and 3 nmol/L Iso. The dotted traces indicate consecutive APs in the same cell.

**Online Table 1.** The effect of hypoxia ± Iso on action potential parameters (model and experiment). Normoxia is PO$_2$ of 150 mmHg. Hypoxia is PO$_2$ of 17 mmHg. All data correspond to cycle length (CL) of 1000 ms. Unless otherwise noted concentrations of Iso were 0.6 nmol/L except for hypoxia + Iso ($I_{Ca-L}$ + $I_{Ks}$) which was 0.5 nmol/L. RMP: resting membrane potential. APP: action potential peak. APD: action potential duration. MRD: maximum rate of depolarization. EAD: early afterdepolarization. $I_{Na-L}$: persistent Na$^+$ current. $I_{Ca-L}$: L-type Ca$^{2+}$ channel current. $I_{Ks}$: slow component of the delayed rectifier K$^+$ current. n = no. of cells. # $P < 0.001$ compared to hypoxia. Statistical significance determined using one way ANOVA, with Bonferroni’s multiple comparison post hoc test.
References


Online Figure I
Online Figure II
Online Figure III
Online Figure IV
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