β-Adrenergic Stimulation Modulates Ryanodine Receptor Ca²⁺ Release During Diastolic Depolarization to Accelerate Pacemaker Activity in Rabbit Sinoatrial Nodal Cells

Tatiana M. Vinogradova, Konstantin Yu. Bogdanov, Edward G. Lakatta

Abstract—It has long been recognized that activation of sympathetic β-adrenoceptors (β-ARs) increases the spontaneous beating rate of sinoatrial nodal cells (SANCs); however, the specific links between stimulation of β-ARs and the resultant increase in firing rate remain an enigma. In the present study, we show that the positive chronotropic effect of β-AR stimulation is critically dependent on localized subsarcolemmal ryanodine receptor (RyR) Ca²⁺ releases during diastolic depolarization (CRDD). Specifically, isoproterenol (ISO; 0.1 μmol/L) induces a 3-fold increase in the number of CRDDs per cycle; a shift to higher CRDD amplitudes (from 2.00±0.04 to 2.17±0.03 F/F₀; P<0.05 [F and F₀ refer to peak and minimal fluorescence]); and an increase in spatial width (from 3.80±0.44 to 5.45±0.47 μm; P<0.05). The net effect results in an augmentation of the amplitude of the local preaction potential subsarcolemmal Ca²⁺ transient that, in turn, accelerates the diastolic depolarization rate, leading to an increase in SANC firing rate. When RyRs are disabled by ryanodine, β-AR stimulation fails to amplify subsarcolemmal Ca²⁺ releases, fails to augment the diastolic depolarization rate, and fails to increase the SANC firing rate, despite preserved β-AR stimulation-induced augmentation of L-type Ca²⁺ current amplitude. Thus, the RyR Ca²⁺ release acts as a switchboard to link β-AR stimulation to an increase in SANC firing rate: recruitment of additional localized CRDDs and partial synchronization of their occurrence by β-AR stimulation lead to an increase in the heart rate. (Circ Res. 2002;90:73-79.)

Key Words: sinoatrial node ▪ β-adrenergic stimulation ▪ ryanodine receptor ▪ local Ca²⁺ release

A robust fight or flight response, largely mediated via abrupt sympathetic nervous input to the heart to increase its beating rate, is an essential component of the vertebrate survival instinct. This β-adrenoceptor (β-AR)–induced increase of the sinoatrial nodal cell (SANC) beating rate had initially been attributed exclusively to modulation of ionic channels located within the surface membrane. Given the more recently recognized critical role of intracellular Ca²⁺ signaling, an important issue arises as to whether the increase in SANC beating rate effected by β-AR stimulation also involves a β-AR effect on intracellular Ca²⁺ dynamics. In this regard, evidence has been presented to suggest that altered Ca²⁺ flux via ryanodine receptors (RyRs) is involved in the β-AR chronotropic effect in frog and guinea pig SANCs. However, the specific mechanisms of RyR involvement in β-AR stimulation-induced increase in the SANC firing rate are unknown. In the present study, we show in single, isolated rabbit sinoatrial (SA) node cells that β-AR stimulation increases the number of RyR-generated CRDDs. This increased likelihood for CRDD occurrence functionally results in their partial synchronization, producing a localized subsarcolemmal Ca²⁺ transient that spreads by Ca²⁺-induced Ca²⁺ release, activating Na⁺-Ca²⁺ exchange current to accelerate the diastolic depolarization, and thus increases the SANC firing rate. Inhibition of RyRs blocks the chronotropic effect of β-AR stimulation, although this maneuver does not prevent the β-AR stimulation-induced increase in L-type Ca²⁺ current (I_{Ca,L}). Thus, modulation of localized CRDD characteristics during pacemaker depolarization is a novel mechanism involved in β-AR modulation of cardiac chronotropy.

Materials and Methods

SA Node Cell Preparations and Electrophysiological Recordings

Single SA node cells were isolated, using protocols approved by our institution’s Animal Care and Use Committee, from rabbit (Harlan Industries, Indianapolis, Ind) hearts as previously described. We studied spindle-shaped SA node cells that exhibited spontaneous contractions in normal Tyrode solution containing 1.8 mmol/L Ca²⁺. Perforated or ruptured patch-clamp techniques were used to record spontaneous action potentials or currents, respectively, with Axopatch-1D patch-clamp amplifier (Axon Instruments). The bath temperature was maintained at 35±0.5°C. For perforated patch-clamp experiments, β-escin (50 μmol/L, Sigma) was added to the pipette solution. For I_{Ca,L} recordings, depolarizing voltage-clamp pulses (300 ms) were applied from a holding potential of −50 mV; 30 μmol/L

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tetradotoxin (TTX) and 4 mmol/L 4-aminopyridine (4-AP) were added to block interfering currents. To minimize interference from rundown, \( I_{\text{Ca,L}} \) was measured every 11 seconds (step to \(-10 \text{ mV}\)), and the effect of isoproterenol (ISO) was expressed as maximal \( I_{\text{Ca,L}} \) amplitude in the presence of ISO normalized to amplitude immediately before ISO addition. For recordings of diastolic depolarization current, a voltage ramp protocol was used from a holding potential of \(-60 \text{ to } -40 \text{ mV}\).

Confocal Imaging of Ca\(^{2+}\) Transients

Cells were placed on the stage of a Zeiss LSM-410 inverted confocal microscope (Carl Zeiss, Inc) and loaded with fluo-3 AM (Molecular Probes). All images were recorded in the line-scan mode (see Figure 4, inset) with the scan line oriented along the long axis of the SANCs close to the sarcosomal membrane. In this mode, the lines are plotted along the cell every 1.39 to 5 ms, and each line is added to the right of the preceding line to form the line-scan image. A trigger signal and a short flash generated by the voltage-clamp protocol were recorded simultaneously by the electrophysiological and imaging systems to allow synchronization of electrophysiological and Ca\(^{2+}\) measurements. Image processing was performed with IDL software (version 5.2, Research Systems, Boulder, Colo). To identify and measure local CRDDs, a customized software program was used that selected CRDDs on the basis of their statistical deviation from the background noise.\(^9\) The amplitude of local CRDDs was expressed as a peak value (\( F \)) normalized to minimal fluorescence (\( F_0 \)); spatial composition (in mmol/L): NaCl 140, KCl 5.4, MgCl\(_2\) 1, HEPES 5, and CaCl\(_2\) 1.8, and glucose 5.5; pH 7.4. Pipette solution for both perforated patch study and diastolic depolarization current recordings contained (in mmol/L): K-gluconate 120, NaCl 5, MgATP 5, and HEPES 5, and KCl 20; pH 7.2. For \( I_{\text{Ca,L}} \) recordings, the bath solution contained the following (in mmol/L): NaCl 140. KCl 5.4, MgCl\(_2\) 1, HEPES 5, CaCl\(_2\) 1.8, and 4-AP 4; pH 7.4. TTX (30 \( \mu \text{mol/L} \)) was added to block interfering currents. To minimize interference from rundown, \( I_{\text{Ca,L}} \) was measured every 11 seconds (step to \(-10 \text{ mV}\)), and the effect of isoproterenol (ISO) was expressed as maximal \( I_{\text{Ca,L}} \) amplitude in the presence of ISO normalized to amplitude immediately before ISO addition. For recordings of diastolic depolarization current, a voltage ramp protocol was used from a holding potential of \(-60 \text{ to } -40 \text{ mV}\).

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Solutions and Materials

For action potential recordings, the bath solution had the following composition (in mmol/L): NaCl 140, KCl 5.4, MgCl\(_2\) 1, HEPES 5, CaCl\(_2\) 1.8, and glucose 5.5; pH 7.4. Pipette solution for both perforated patch study and diastolic depolarization current recordings contained (in mmol/L): K-glucuronate 120, NaCl 5, MgATP 5, HEPES 5, and KCl 20; pH 7.2. For \( I_{\text{Ca,L}} \) recordings, the bath solution contained the following (in mmol/L): NaCl 117, TEA-Cl 20, CsCl 5.4, MgCl\(_2\) 1, HEPES 5, CaCl\(_2\) 1.8, and 4-AP 4; pH 7.4. TTX (30 \( \mu \text{mol/L} \)) was added to the bath solution before the experiment. The pipette solution contained the following (in mmol/L): NaCl 10, TEA-Cl 20, CsCl 110, EGTA 10, MgATP 5, and HEPES 10; pH 7.2.

Statistical Analysis

Data are presented as mean±SEM. The statistical significance of effects was evaluated by Student’s \( t \) test and ANOVA when appropriate. A value of \( P<0.05 \) was considered statistically significant.

Results and Discussion

Efficiency of \( \beta\)-AR Stimulation Is Suppressed When RyRs Are Disabled

Table 1 and Figure 1A show that the \( \beta\)-adrenergic agonist ISO significantly increases spontaneous SANC firing rate. This effect is reversible during washout and occurs via \( \beta\)-ARs, as the application of the \( \beta\)-AR antagonist propranolol (2 \( \mu \text{mol/L} \)) during ISO stimulation reverses the increase in the firing rate (\( n=3 \), data not shown). Table 1 also lists the control parameters of SANC action potentials, which are highly comparable to those reported previously.\(^1,13\)

![Image](http://circres.ahajournals.org/)

**Figure 1.** Effects of \( \beta\)-adrenergic agonist ISO (0.3 \( \mu \text{mol/L} \)) on the firing rate of control SANCs (A) and after block (B) of SR Ca\(^{2+}\) release channels by ryanodine (3 \( \mu \text{mol/L} \)). A and B. Recordings of action potentials in representative SANCs. In panel A, the firing rate was 174 bpm; after ISO, it increased to 244 bpm. In the cell in panel B, the firing rate before and after ryanodine (Ry, 3 \( \mu \text{mol/L} \)) was 146 and 86 bpm, respectively. ISO increased the firing rate in this cell to only 91 bpm. C, Time course of the average increase in firing rate by 0.3 \( \mu \text{mol/L} \) of ISO in control cells (\( n=3 \)) and in those pretreated with 3 \( \mu \text{mol/L} \) of ryanodine (\( n=3 \)).

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**TABLE 1.** Effect of ISO (1 \( \mu \text{mol/L} \)), Ryanodine (3 \( \mu \text{mol/L} \)), and a Combination of Both Drugs on SANC Action Potential Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=15)</th>
<th>ISO (n=10)</th>
<th>Ry (n=9)</th>
<th>Ry+ISO (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate, bpm</td>
<td>156.5±10.4</td>
<td>205.0±5.3*</td>
<td>93.7±5.8*</td>
<td>98.0±3.6†</td>
</tr>
<tr>
<td>APA, mV</td>
<td>96.4±2.7</td>
<td>95.2±3.2</td>
<td>107.9±2.2*</td>
<td>95.9±5.0</td>
</tr>
<tr>
<td>OS, mV</td>
<td>38.6±1.4</td>
<td>36.6±2.6</td>
<td>50.3±1.4*</td>
<td>41.0±3.4</td>
</tr>
<tr>
<td>MDP, mV</td>
<td>-57.8±1.4</td>
<td>-58.6±1.7</td>
<td>-57.6±2.1</td>
<td>-54.9±2.2</td>
</tr>
<tr>
<td>DD, mV/s</td>
<td>70.9±4.6</td>
<td>101.8±10.9*</td>
<td>45.3±3.0*</td>
<td>51.7±4.3†</td>
</tr>
<tr>
<td>APD(_{50}), ms</td>
<td>120.1±7.9</td>
<td>95.2±8.1</td>
<td>183.2±21.9*</td>
<td>166.4±23.2†</td>
</tr>
</tbody>
</table>

 APA indicates action potential amplitude; OS, overshoot; MDP, maximal diastolic potential; DD, rate of diastolic depolarization; APD\(_{50}\), 50% action potential duration; and \( n \), number of cells.

*Significantly different (\( P<0.05 \)) vs control.
†Significantly different (\( P<0.05 \)) vs ISO.
To determine whether the mechanism responsible for this β-adrenergic acceleration of SANC firing depends on Ca\(^{2+}\) release from RyRs, we used ryanodine to functionally disable RyRs.\(^{16}\) Consistent with previous results,\(^{3,6}\) ryanodine decreased the firing rate of SANCs in a dose-dependent manner (EC\(_{50}\) 3 μmol/L) and at 30 μmol/L of ryanodine completely abolished spontaneous excitations.\(^{8}\) Changes in SANC action potential parameters induced by ryanodine (see Table 1) are similar to those reported previously for rabbit SANCs.\(^{5,6}\) Application of ISO in the presence of ryanodine (3 μmol/L) induced only a minor increase in the rate of spontaneous firing (Figure 1B) and this small effect was transient (Figure 1C). Compared with control, the ability of ISO (1 μmol/L) to accelerate the diastolic depolarization rate in the presence of ryanodine was decreased by approximately 3-fold (see Table 1). The average concentration response of the relative change in SANC firing rate have not been demonstrated.

**β-Adrenergic Modulation of CRDD Parameters**

To define the specific subcellular spatiotemporal characteristics of RyR Ca\(^{2+}\) release and to determine how β-AR stimulation modulates this release to enhance SANC firing, we used confocal microscopy to measure changes in Ca\(^{2+}\) beneath the sarcolemmal membrane. Prior studies in cat atrial pacemaker cells\(^{7}\) and rabbit SANCs\(^{8}\) have noted the occurrence of local subsarcolemmal Ca\(^{2+}\) releases during the latter half of the diastolic depolarization, i.e., preceding the action potential upstroke. Figures 3A and 3C demonstrate such CRDDs in rabbit SANCs, most of which occurred during the second half of the diastolic depolarization. The representative image in Figure 3B indicates that β-AR stimulation increases the likelihood for CRDDs to occur, i.e., recruits more Ca\(^{2+}\) releases, and increases their brightness. Figure 4 compares the average number of CRDDs during each cycle (Figure 4A), their amplitude (Figure 4B), spatial width (Figure 4C), and duration (Figure 4D) before and after β-AR stimulation. In control conditions, although the mean CRDD duration was the same as in ventricular myocytes, the amplitude and width measured at half-maximum was about twice as large as in ventricular myocytes,\(^{17-19}\) which suggests that each CRDD in SANCs involves more RyR release units than in ventricular myocytes. After β-AR stimulation, there is a 3-fold increase in number of CRDDs per cycle as well as a shift to higher amplitudes and greater width (see Figure 4). There is also a
Effect of β-AR Stimulation on Diastolic Depolarization, Ca2+ Currents, and \( I_{\text{f}} \)

To delineate the ionic mechanisms underlying β-AR–induced Ca2+ release-mediated modulation of SANC pacemaker activity, we simulated the action potential diastolic depolarization by a voltage ramp protocol (Figure 5A, bottom), which followed six preconditioning pulses to ensure steady-state loading of the SR Ca2+. If CRDDs were to activate net inward current, their partial synchronization by β-AR stimulation would augment the slope of the diastolic depolarization; conversely, inhibition of such Ca2+ releases would be expected to decrease net inward current and reduce the diastolic depolarization slope. In control, ISO significantly increased this current (Figure 5B). Ryodin, and per se, reduced average diastolic depolarization current from 0.93±0.13 to 0.48±0.16 pA/pF (n=6, P<0.05). After ryodinetreatment, ISO did not produce a significant increase in the diastolic depolarization current (Figure 5B) or in the SANC firing rate (Figure 2). The most likely candidate responsible for this ryodine-sensitive inward current during the SANC diastolic depolarization is the Na+-Ca2+ exchanger current (\( I_{\text{Na},\text{Ca}} \)), which has been reported to contribute significantly to automaticity of SANCs.2,8,20,21 That this current was completely abolished after substituting Na+ for Li+ in the bath solution provides further evidence that this current is \( I_{\text{Na},\text{Ca}} \).

It is widely recognized that the positive chronotropic effect of β-AR stimulation in SANCs is accompanied by an increase in the \( I_{\text{Ca},\text{L}} \) amplitude.1 To determine whether the aforementioned effects of ryodine to reduce the ISO-induced increase in the inward current during the pacemaker depolarization concomitantly affects \( I_{\text{Ca},\text{L}} \), we measured effects of ISO on \( I_{\text{Ca},\text{L}} \) in the presence and absence of ryodine.

Ryodinetreatment, per se, had no significant effect on the average amplitude of \( I_{\text{Ca},\text{L}} \) (Table 2). Likewise, ryodinetreatment had no significant effect on the ISO-induced augmentation of the \( I_{\text{Ca},\text{L}} \) amplitude (Table 2 and Figure 5D). Thus, even when β-AR stimulation substantially increases Ca2+ influx via augmentation of \( I_{\text{Ca},\text{L}} \) amplitude, the net positive chronotropic effect of ISO is suppressed when RyRs are disabled and CRDDs from RyRs are inhibited by ryodine (Figures 1C, 2, and 3E). In other words, in the absence of intact RyR function, a substantial β-AR stimulation-induced increase of \( I_{\text{Ca},\text{L}} \) in the presence of ryodine is not sufficient to increase the inward current that underlies SANC diastolic depolarization (Figure 5B) or to

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**Figure 4.** Microscopic properties of CRDDs in SANCs and their modulation by β-adrenergic stimulation. Average data from 4 cells obtained before (white bars, 18 local CRDDs) and after superfusion with ISO (0.1 μmol/L, black bars, 54 local CRDDs), with the scanned line oriented parallel to the long axis of the cell just beneath the sarcolemma (see cartoon). After β-AR stimulation, the average occurrence of CRDDs increased 3-fold, from 0.61±0.13 per cycle to 1.71±0.37 per cycle (A). B, Amplitude of CRDDs indexed by F/F0, where F and F0 refer to peak and minimal fluorescence; in control, this was 2.00±0.04 vs 2.17±0.03 in the presence of ISO. C, Spatial size, indexed by FWHM; in control, this was 3.80±0.44 vs 5.45±0.48 μm in the presence of ISO. D, Duration of CRDDs, indexed by T50; in control, this was 41.33±4.50 vs 45.40±4.77 ms in the presence of ISO (P>0.05). *P<0.05.

**Figure 5.** Effects of RyR inhibition on the ISO potentiation of diastolic depolarization current (A) and \( I_{\text{Ca},\text{L}} \) (B) in SANCs. A and C, Voltage protocols and original current recordings for the simulated diastolic depolarization (DD) current and \( I_{\text{Ca},\text{L}} \), respectively. B, Relative increase in mean DD current amplitude by 1 μmol/L of ISO before (n=9) and after block of RyR with 3 μmol/L of ryodine (n=4). D, Relative increase in mean value of the \( I_{\text{Ca},\text{L}} \) by 1 μmol/L of ISO before (n=6) and after 10 μmol/L of ryodine pretreatment (n=8). *P<0.05.
increase SANC beating rate. Thus, amplification of local increase of subsarcolemmal Ca$^{2+}$ during pacemaker depolarization, which accelerates the diastolic depolarization slope via amplification of inward current, is an important link in the cascade of mechanisms that underlies the β-adrenergic modulation of SANC pacemaker activity.

The requirement of intact RyRs for the β-AR stimulation-induced amplification of CRDDs may be attributable to a direct phosphorylation of the RyRs or to an effect of β-ARs to increase the SR Ca$^{2+}$ load. To test the latter possibility, we applied a pulse of caffeine (20 mmol/L, 1 second), which rapidly empties the SR Ca$^{2+}$ store (Figure 6). After 1 minute of ISO superfusion, there was a significant (P<0.05) increase in the SR Ca$^{2+}$ load by 31% (Figure 6). Thus, the increase in CRDDs by β-AR stimulation could be partly attributable to an increase in RyR release flux due to an increase in the SR Ca$^{2+}$ load.

The increase in SR Ca$^{2+}$ load and increased RyR Ca$^{2+}$ release flux triggered by $I_{C_{a-L}}$ also affected $I_{C_{a-L}}$ decay kinetics. The fast time constant of $I_{C_{a-L}}$ decay, which reflects Ca$^{2+}$-dependent inactivation, was significantly increased after ryanodine pretreatment, whereas the slow time constant remained unchanged (Table 2). This result is similar to that of prior observations of the effect of ryanodine on $I_{C_{a-L}}$ inactivation in rat ventricular myocytes and human atrial myocytes and is consistent with the interpretation that Ca$^{2+}$ influx through L-type channels and $I_{C_{a-L}}$-triggered Ca$^{2+}$ release from SR accelerate time-dependent inactivation of $I_{C_{a-L}}$.

In the present study, the effect of ryanodine to decrease Ca$^{2+}$ release from the SR in response to an $I_{C_{a-L}}$, of a given amplitude likely reflects a reduction in RyR Ca$^{2+}$ release flux, a widely recognized effect of ryanodine in ventricular cells. That ISO accelerates the fast component of $I_{C_{a-L}}$ decay (Table 2) in spite of the presence of 10 mmol/L EGTA in our pipette solution is consistent with the idea that EGTA is a slow Ca$^{2+}$ buffer and cannot effectively buffer Ca$^{2+}$ released from the SR near the mouth of L-type Ca$^{2+}$ channel. In the presence of ryanodine, ISO did not affect the kinetics of $I_{C_{a-L}}$ decay (Table 2). This is consistent with the idea that ryanodine reduces RyR Ca$^{2+}$ release flux in the presence of ISO.

It is not clear whether enhanced CRDDs via RyRs during β-AR activation occur spontaneously or are triggered. Spontaneous, localized, diastolic SR Ca$^{2+}$ release in ventricular myocytes causes spontaneous sarcolemmal diastolic depolarization mainly because of Ca$^{2+}$ modulation of Na$^{+}$-Ca$^{2+}$ exchanger, and when diastolic depolarization amplitude reaches threshold, it elicits a spontaneous action potential. The increase in cellular SR Ca$^{2+}$ load in ventricular myocytes by β-AR stimulation markedly exaggerates this phenomenon. This mechanism of abnormal automaticity in ventricular myocytes may underlie normal automaticity in SANCs. Alternatively, as all CRDDs in SANCs occur during the second half of the diastolic depolarization, it is possible that sarcolemmal ion channel activation during this period evokes CRDDs. A likely candidate of β-AR stimulation-induced augmentation of CRDDs would be L-type Ca$^{2+}$ channels activated during SANC diastolic depolarization.

Changes in other ionic currents that underlie SANC diastolic depolarization might also be involved in the triggering of CRDDs and their modulation by β-AR stimulation. It has been observed in latent cat atrial pacemaker cells that a blockade of T-type Ca$^{2+}$ current with Ni$^{2+}$ (50 μmol/L) induces a dramatic (230%) prolongation of spontaneous cycle length compared with control. To define whether the beating rates and CRDDs in rabbit SANCs in the present study involve T-type Ca$^{2+}$ current, we exposed SANCs to 50 μmol/L Ni$^{2+}$. In contrast to the prior observation in cat latent atrial pacemaker cells, Ni$^{2+}$ induced only a 16% increase in the spontaneous cycle length of rabbit SANCs (from 440±20 to 510±63 ms, n=5). This result is consistent with a previous study in rabbit SANCs in which abolition of T-type Ca$^{2+}$ current with 40 μmol/L of Ni$^{2+}$ only slightly (14%) increased the spontaneous cycle length. Moreover, in our experiments, 50 μmol/L of Ni$^{2+}$ did not decrease the number of

**TABLE 2. Change in $I_{C_{a-L}}$ Amplitude and Kinetics of Inactivation in Rabbit SANCs Before and After Application of ISO (1 μmol/L), Ryanodine (10 μmol/L), and a Combination of the Two Drugs**

<table>
<thead>
<tr>
<th></th>
<th>$I_{C_{a-L}}$ Amplitude, pA/pF</th>
<th>$\tau_f$, ms</th>
<th>$\tau_s$, ms</th>
<th>[A$_i$/(A$_i$+A$_o$)]%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.8±1.3 (n=6)</td>
<td>6.6±0.2 (n=12)</td>
<td>59.0±2.4 (n=12)</td>
<td>66.5±1.4 (n=12)</td>
</tr>
<tr>
<td>+ISO</td>
<td>24.0±2.2* (n=6)</td>
<td>5.3±0.3* (n=6)</td>
<td>59.8±3.9 (n=6)</td>
<td>65.3±1.7 (n=6)</td>
</tr>
<tr>
<td>+Ry</td>
<td>12.9±1.1 (n=8)</td>
<td>8.5±0.3* (n=8)</td>
<td>55.2±3.8 (n=8)</td>
<td>65.3±2.1 (n=8)</td>
</tr>
<tr>
<td>+Ry+ISO</td>
<td>21.9±1.1* (n=8)</td>
<td>8.6±0.7* (n=8)</td>
<td>59.7±8.1 (n=8)</td>
<td>66.4±2.2 (n=8)</td>
</tr>
</tbody>
</table>

$\tau_f$ and $\tau_s$ indicate fast and slow time constants of $I_{C_{a-L}}$ inactivation, respectively; n, number of cells.

*Significantly different (P<0.05) vs control.

![Figure 6. Effects of β-AR stimulation on the SR Ca$^{2+}$ content in SANCs. Spontaneous Ca$^{2+}$ transients and caffeine-induced SR Ca$^{2+}$ release in the same cell in control conditions (A) when the firing rate was 131 bpm and in the presence of 0.1 μmol/L ISO (B) when the firing rate increased to 144 bpm. B, Effect of a rapid application of caffeine into the cell in the presence of ISO. C, Average data on the caffeine-induced increase in SR Ca$^{2+}$ release of the initial rapid component (arrow) indexed by F/F$_{o}$ in a control group of cells (n=7) and group of cells (n=7) subjected to 1 minute of superfusion with ISO. P<0.05.](http://circres.ahajournals.org/DownloadedFrom)
spontaneous CRDDs per cycle (in control 1.0±0.1 per cycle versus 1.0±0.3 per cycle in Ni²⁺, n=4). Thus, different perspectives on the role of T-type Ca²⁺ current in CRDDs, diastolic depolarization, and beating rate are gleaned from experiments in different cell types. That T-type Ca²⁺ current appears to play a more important role in cat atrial latent pacemaker activity than in rabbit SANC primary pacemaker activity might be explained on the basis of a more negative maximum diastolic potential in the former than the latter. To determine a role of T-type Ca²⁺ current regulation in β-AR acceleration of SANC beating rate in the present study, we applied 50 μmol/L of Ni²⁺ after β-AR stimulation. ISO (0.1 μmol/L) increased the beating rate from 108±7 bpm in control to 144±5 bpm with ISO, n=3. Ni²⁺, applied after ISO, induced only a 10% decrease in the beating rate, to 139±6 bpm, n=3. Thus, under conditions of the present experiments, T-type Ca²⁺ current likely has, at best, a minor role in β-AR acceleration of rabbit SANC beating rate, a conclusion consistent with that of a prior study that failed to demonstrate an effect of ISO on T-type Ca²⁺ current in rabbit SANCs.

To define the involvement of the hyperpolarization-activated current, Iₖ, in β-AR acceleration of SANC beating rate, we applied 2 mmol/L of Cs⁺ before and during β-AR stimulation. Cs⁺ alone induced a 5.2±0.3% (n=5) decrease in beating rate from 205±6 bpm in control to 194±5 bpm with Cs⁺, consistent with a prior observation in primary pacemaker cells. In the presence of ISO (1 μmol/L), Cs⁺ induced a 7.5±2.6% (n=4) decrease of the SANC beating rate from 225±18 bpm to 208±17 bpm with Cs⁺. Thus, under the present experimental conditions, the contribution of Iₖ to β-AR acceleration of rabbit SANC beating rate did not exceed 10%.

Activation of other ionic currents as a potential mechanism for β-AR stimulation to increase in CRDDs and beating rate in SANCs may be excluded. β-AR–operated chloride current is not involved in the effect of β-AR stimulation, as this current is absent in rabbit SANCs. Although a small change in the amplitude of deactivation kinetics of the delayed rectifier K⁺ current might theoretically contribute to the positive chronotropic effect of ISO, a significant change in Iₖ is unlikely because the maximum diastolic potential was not affected by ISO (see Table 1), and the slope of diastolic depolarization was altered only just before the action potential upstroke (Figure 1A).

In summary, the present results show, for the first time, that β-AR stimulation increases the likelihood of occurrence of localized Ca²⁺ release in the subsarcolemmal space during the pacemaker depolarization and augments its amplitude and spatial dimension, ie, β-AR recruits additional local RyR Ca²⁺ release and partially synchronizes its occurrence. This spatiotemporal synchronization of CRDDs augments the inward current during the diastolic depolarization, which, in turn, accelerates the diastolic depolarization rate, leading to an increase in SANC firing rate. In the presence of ryanodine, which disables normal RyR function and depletes the SR Ca²⁺ store, β-AR stimulation fails to amplify local CRDDs, fails to augment the diastolic depolarization, and fails to increase the SANC firing rate (Figures 1 and 2). Thus, the RyR Ca²⁺ release flux acts as a switchboard that links β-AR stimulation to an increase in SANC firing rate. β-AR modulation of localized subsarcolemmal RyR-generated Ca²⁺ release characteristics during the diastolic depolarization demonstrated in the present study is a novel mechanism to explain β-AR modulation of cardiac chronotropy.

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β-Adrenergic Stimulation Modulates Ryanodine Receptor Ca\textsuperscript{2+} Release During Diastolic Depolarization to Accelerate Pacemaker Activity in Rabbit Sinoatrial Nodal Cells

Tatiana M. Vinogradova, Konstantin Yu. Bogdanov and Edward G. Lakatta

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