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Membrane Potential Fluctuations Resulting From Submembrane Ca\(^{2+}\) Releases in Rabbit Sinoatrial Nodal Cells Impart an Exponential Phase to the Late Diastolic Depolarization That Controls Their Chronotropic State

Konstantin Y. Bogdanov,* Victor A. Maltsev,* Tatiana M. Vinogradova, Alexey E. Lyashkov, Harold A. Spurgeon, Michael D. Stern, Edward G. Lakatta

Abstract—Stochastic but roughly periodic LCRs (Local subsarcolemmal ryanodine receptor–mediated Ca\(^{2+}\) Releases) during the late phase of diastolic depolarization (DD) in rabbit sinoatrial nodal pacemaker cells (SANCs) generate an inward current \(I_{\text{NCX}}\) via the Na\(^+\)/Ca\(^{2+}\) exchanger. Although LCR characteristics have been correlated with spontaneous beating, the specific link between LCR characteristics and SANC spontaneous beating rate, ie, impact of LCRs on the fine structure of the DD, have not been explicitly defined. Here we determined how LCRs and resultant \(I_{\text{NCX}}\) impact on the DD fine structure to control the spontaneous SANC firing rate. Membrane potential \((V_m)\) recordings combined with confocal Ca\(^{2+}\) measurements showed that LCRs impart a nonlinear, exponentially rising phase to the DD later part, which exhibited beat-to-beat \(V_m\) fluctuations with an amplitude of approximately 2 mV. Maneuvers that altered LCR timing or amplitude of the nonlinear DD (ryanodine, BAPTA, nifedipine or isoproterenol) produced corresponding changes in \(V_m\) fluctuations during the nonlinear DD component, and the \(V_m\) fluctuation response evoked by these maneuvers was tightly correlated with the concurrent changes in spontaneous beating rate induced by these perturbations. Numerical modeling, using measured LCR characteristics under these perturbations, predicted a family of local \(I_{\text{NCX}}\) that reproduced \(V_m\) fluctuations measured experimentally and determined the onset and amplitude of the nonlinear DD component and the beating rate. Thus, beat-to-beat \(V_m\) fluctuations during late DD phase reflect the underlying LCR/\(I_{\text{NCX}}\) events, and the ensemble of these events forms the nonlinear DD component that ultimately controls the SANC chronotropic state in tight cooperation with surface membrane ion channels. (Circ Res. 2006;99:979-987.)

Key Words: membrane potential ■ Na\(^+\)/Ca\(^{2+}\) exchange ■ ryanodine receptor ■ sarcoplasmic reticulum ■ sinoatrial node

The spontaneous diastolic depolarization (DD) of sino-atrial nodal cells underlies their rhythmic activity. It results from the instantaneous change in the ensemble of ionic currents expressed by sinoatrial nodal pacemaker cells (SANCs), which have been studied extensively using the voltage-clamp technique.\(^1,2\) The major currents thought to be involved in diastolic depolarization during normal spontaneous activity in the SANCs are as follows: rapid and slow delayed rectifier K\(^+\) currents; L- and T-type Ca\(^{2+}\) currents; \(I_c\); a background current; and an Na\(^+\)/Ca\(^{2+}\) exchange (NCX) current \((I_{\text{NCX}})\).

Recent studies have demonstrated the occurrence\(^3\) and relevance\(^4–7\) of submembrane LCRs (Local subsarcolemmal Ca\(^{2+}\) Releases) via ryanodine receptors (RyRs) to the spontaneous firing rate of SANCs. Specifically, spontaneous, voltage-independent,\(^7\) roughly periodic LCRs in SANCs are generated because of an enhanced Ca\(^{2+}\) cycling by sarcoplasmic reticulum (SR) proteins, which, in contrast to ventricular myocytes, are highly phosphorylated at the basal state.\(^8\) The Ca\(^{2+}\) release activation phase of the cycling occurs during the later, nonlinear part of diastolic depolarization and produce an inward current via activation of NCX.\(^5,9\) Whereas the resultant depolarization from the local NCX activation is likely the event that links individual LCR to DD acceleration, quantitative characterization of how LCRs, in cooperation with the established membrane-delimited pacemaker mechanisms, specifically control the fine structure of the DD has not been reported.

The present study used a combination of experimental and numerical approaches to identify depolarizations induced by the LCR-activated, local NCX currents and to determine how these depolarizations impact on the fine structure of the DD.
to control the spontaneous firing rate under a variety of conditions that we had previously shown to alter LCR timing and/or amplitude (ie, ryanodine, BAPTA, nifedipine, isoproterenol [ISO]). The results of our study show that LCR-induced NCX currents link LCRs to the spontaneous beat-to-beat membrane potential ($V_{m}$), the ensemble of which imparts an exponentially rising phase to the late nonlinear DD fine structure, followed by $I_{CaL}$ activation and action potential (AP) upstroke, thus linking LCRs to the cycle length and hence spontaneous SANC beating rate.

Materials and Methods

Preparation and Imaging of Cells
Single rabbit SANCs were isolated as described previously,10 using protocols approved by the Animal Care and Use Committee of our institution. Cells were loaded with fluo-3 AM (Molecular Probes), and a LSM-410 microscope (Carl Zeiss Inc) was used to image Ca$^{2+}$ in the line scan mode. Image processing was performed with IDL software (Research Systems, Boulder, Co), and Ca$^{2+}$ release was expressed as fluo-3 fluorescence ($F$) normalized to its minimal value ($F_{0}$). Cells chosen for the study had a spindle-like or spider-like shape.

Electrophysiological Recordings
A perforated patch clamp was used to record membrane potential by an Axopatch 2D amplifier (Axon Instruments, Foster City, Calif), operating in the current-clamp mode. Pipettes were filled with (in mmol/L) potassium gluconate 120, KCl 20, NaCl 5, HEPES 5, MgATP 5, and (in µmol/L) β-escin 50 (pH 7.2). The extracellular bathing solution contained (in mmol/L) NaCl 140, KCl 5.4, MgCl$_2$ 1, HEPES 5, CaCl$_2$ 1.8, and glucose 5.5 (pH 7.4). All experiments were performed at 35°C.

Data were acquired using pClamp software (Axon Instruments). Spontaneous beating of SANCs was continuously recorded for at least 7 to 10 minutes in control conditions as well as during drug (ISO, BAPTA-AM, nifedipine, cesium, and ryanodine) application. Drugs, dissolved in the bath solution, were usually applied for 4 to 5 minutes before recordings of APs demonstrating effect of drug were taken for the analysis.

Analysis of DD Shape and Its Beat-to-Beat Fluctuations
We analyzed the fine structure DD by calculating the mean DD slope, the amplitude of nonlinear DD component (NDDC), and the AP take-off potential as well as beat-to-beat fluctuations, by calculating the mean DD and variance estimates (Figure 1A; also see the online data supplement for details, available at http://circres.ahajournals.org).

Computer Simulations
A numerical primary SANC model9 was used to simulate SANC electrophysiology and LCR stochastics (see the online data supplement for details).

Statistics
Data are expressed as mean±SEM. Statistical significance was determined using Students $t$ test (significance level, $P<0.05$).

Results

Beat-to-Beat Fluctuations in Pacemaker Potential
Spontaneous APs of rabbit SANCs recorded continuously in control conditions (Figure 1B; Table II in the online data supplement) were similar to those reported in previous studies.$^{1,10,11}$ The DD consists of 2 components: an initial linear component, and a later, nonlinear component (Figure 1 and the online data supplement). Under control conditions, the time from the prior maximum diastolic potential (MDP) to the onset of the nonlinear component in 31 SANCs averaged 145±8 ms (NDDC delay time in supplemental Table II). We observed beat-to-beat membrane potential fluctuations in the NDDC (Figure 1C) and quantified them during the 100-ms segment preceding AP upstroke as the...
residual variance in each beat after the group mean DD had been subtracted (Figure 1D and the online data supplement).

The superimposition of 7 individual DDs of the beats in the representative cell (Figure 1C) and the mean of DD of 7 consecutive beats (bold curve) reveals that beat-to-beat fluctuations in DD begin to occur approximately 70 ms before the next AP upstroke and increase with time. Figure 1D shows in more detail the membrane potential fluctuations about the mean DD preceding each of the 7 APs. Figure 1E shows the time course of the total ensemble variance (see the online data supplement for calculation details) in the cell during control conditions. For the 50-ms segment of the NDDC analyzed, the mean value of DD fluctuations was 1.07±0.06 mV (n=31).

Local Ca\(^{2+}\) Releases Are Linked to the Nonlinear Diastolic Depolarization Phase

Figure 2A shows the membrane potential recording of a representative SANCs superimposed on a line-scan image of [Ca\(^{2+}\)]i with the scanned line positioned close to the sarcolemmal membrane parallel to the longitudinal axis of the cell (inset in top left). Note, that subsarcolemmal LCRs occur during the nonlinear DD, peaking before the next AP upstroke. The Ca\(^{2+}\) releases have widths of 2 to 8 μm. Therefore, some are small waves, but some appear like sparks (ie, events with no evident Ca\(^{2+}\) propagation from one release site to another); however, they have a larger size (2 to 5 times) compared with sparks in ventricular myocytes.5

In Figure 2B, the average time course of the LCR occurrence is superimposed on that of DD variance. The novel finding here is that the time course of LCR evolution during DD and that of DD variance are virtually superimposable. The DD variance peaks at 1.51±0.15 mV\(^2\) (n=31) at −40 ms, ie, when the nonlinear component contributes significantly to DD (see Figure 2C).

DD Fine Structure and Cycle Length Responses to Maneuvers Known to Alter LCRs

Figure 3A, left, shows an averaged time course of DD variance (n=6); in control, DD variance increases between 80 and 40 ms before the subsequent AP and is markedly decreased by ryanodine. On average (Figure 3A, right), ryanodine at this concentration reduced the amplitude of the DD fluctuations for the 50-ms segment preceding AP upstroke by 60% (n=6). The profound inhibition of DD variance during ryanodine exposure strongly suggests that the DD fluctuations are caused by stochastic Ca\(^{2+}\) releases via the RyRs. It is important to note that in response to ryanodine the amplitude of the NDDC also decreased by approximately 50%, the onset of the nonlinear DD was delayed by approximately 2-fold, and the cycle length increased nearly 2-fold (supplemental Table II).

To provide additional evidence that DD variance is caused by LCRs, we exposed SANCs to a fast Ca\(^{2+}\) chelator, BAPTA-AM (5 μmol/L). Figure 3B shows that BAPTA strikingly decreases DD variance. On average (Figure 3B, right), the DD fluctuations for the 50-ms segment preceding AP upstroke were decreased by approximately 50%. Of note, BAPTA also reduced the amplitude of the NDDC by approximately 50%, delayed its onset by 3.5-fold, and increased the cycle length by nearly 3-fold (supplemental Table II).

We have previously shown that LCR characteristics vary with the extent of the SR Ca\(^{2+}\) load and that voltage-dependent Ca\(^{2+}\) current is required for Ca\(^{2+}\) influx to load the SR to fuel LCRs.7 Thus, DD variance caused by stochastic LCRs would be expected to vary with changes in voltage-dependent Ca\(^{2+}\) influx during the prior AP plateau. To reduce Ca\(^{2+}\) influx, we used the L-type Ca\(^{2+}\) channel blocker nifedipine (supplemental Table II). As was the case for ryanodine and BAPTA, 100 nmol/L nifedipine suppressed DD fluctuations (by ~50%, from 1.11±0.07 mV to 0.59±0.04 mV), reduced the amplitude of the NDDC (by ~25%), delayed its onset (by 85%), and prolonged the cycle length by 80% (supplemental Table II).

The β-adrenergic receptor agonist, ISO increases Ca\(^{2+}\) influx and has previously been shown to increase LCR frequency and size in rabbit SANCs.6 Figure 3C, left, shows that ISO increases the DD variance. On average (Figure 3C,
right), ISO increased the DD fluctuations over a 50-ms interval preceding the AP upstroke by 40%. Notably, ISO also increased the amplitude of the NDDC by 20%, reduced its time of onset by 30%, and reduced the cycle length by 30% (supplemental Table II).

It has been proposed that the hyperpolarization-activated current $I_f$ plays an important role in the control of automaticity, but the physiological impact of $I_f$ in the generation of the DD has been and continues to be a matter of debate. The contribution of $I_f$ to the amplitude of the NDDC and its variance in SANCs was assessed by exposure to 2 mmol/L cesium, an $I_f$ blocker.

As shown in supplemental Table II, on average, Cs had a significant, but small (5%), negative chronotropic effect under the present conditions, and, in contrast to the other potent chronotropic interventions tested above (BAPTA, ryanodine, nifedipine, and ISO), Cs had no substantive effect on nonlinear DD amplitude, delay (supplemental Table II), or on DD fluctuations.

Nonlinear DD Fine Structure Predicts the Chronotropic State

Figure 4A schematically summarizes the average effect of the aforementioned chronotropic interventions on the onset and amplitude of the NDDC and spontaneous cycle length. Because the DD noise may include a “background” noise related to the recording apparatus, as well as to some electrophysiological processes other than of LCR origin, we characterized the response to chronotropic interventions as the change in noise parameters from the control state. Figure 4B demonstrates the relationship of changes in the NDDCs to

Figure 3. Maneuvers that affect the SR Ca$^{2+}$ cycling and beating rate affect membrane potential noise during the nonlinear DD. Left traces indicate average of DD variance. The average amplitude of DD fluctuations (right panel) was calculated for the 50-ms segment preceding AP upstroke in 4 to 12 cells. A, Effect of 3 μmol/L ryanodine on DD variance (exposure time, 4 minutes; n=6). B, Average effect of 5 μmol/L BAPTA-AM on DD variance in SANCs (n=4). C, ISO (1 μmol/L) increases nonlinear DD variance (n=12). See supplemental Table II for additional effects of these maneuvers, and Figure 4 for correlation among changes in DD noise and amplitude and changes in beating rate affected by these maneuvers.

Figure 4. Relationships among the SANC beating rate, the NDDC, and change in DD fluctuations in response to Ca$^{2+}$-dependent chronotropic interventions. A, Schematic superimposition of spontaneous APs synchronized by ending of previous one at control, after ISO, ryanodine, BAPTA, and nifedipine; arrows show amplitudes of NDDC; shaded area, linear DD component; dotted line corresponds to −20 mV level, where the end of DD was set. The mean level of DD fluctuations was evaluated in the 50-ms segment preceding AP upstroke. B, Changes in DD fluctuations and NDDC amplitude versus changes in beating rate with respect to chronotropic interventions. C, Mechanism of LCR control of the SANC chronotropic state. SANC operation involves a tight cooperation of both membrane and internal Ca$^{2+}$ cycling proteins schematically presented by the 2 interacting loops of membrane channel function cycling and intracellular Ca$^{2+}$ cycling.
changes in the spontaneous beating rate in response to the aforementioned perturbations. Note the strong positive correlations between changes in the beating rate, changes in the mean amplitude of the NDDC, and changes in DD fluctuations.

**Numerical Estimation of NCX Current and DD Disturbance Evoked by an Individual LCR**

A central hypothesis of the origin of DD fluctuations is that they are caused by $I_{NCX}$ fluctuations evoked by stochastically occurring LCRs (Figure 4C). These $I_{NCX}$ fluctuations and related membrane depolarizations were evaluated numerically. Our novel numerical model (online data supplement) predicted that an LCR within submembrane space during DD produces $I_{NCX}$ of approximately $-0.27$ pA, which results in a membrane potential response of approximately $0.17$ mV at the LCR (temporal) center (Figure 5). Also note that a single LCR occurrence results in earlier action potential firing with a phase shift being approximately $1$ ms.

**$I_{NCX}$ Links LCRs to DD Fine Structure and SANC Chronotropic State**

We simulate $I_{NCX}$ generated by an ensemble of stochastic LCRs to determine whether $I_{NCX}$ could provide a mechanistic link between LCRs and the observed DD noise, NDDC, and ultimately the spontaneous beating rate. Figure 6A shows that LCRs indeed control the simulated spontaneous beating rate of our SANC model throughout the entire physiological range. When LCRs enhance with ISO, the beating rate substantially increases from $134$ bpm to $194$ bpm (“ISO” panel). When LCRs cease, the beating rate substantially drops from $134$ bpm to $91$ bpm (“No LCRs” panel), thus simulating the ryanodine effect.

The chronotropic effects were mainly mediated by significant (~2-fold) changes in diastolic $I_{NCX}$ (Figure 6B), which were accompanied by respective DD fluctuation changes (Figure 7A and 7B). In case of ISO, this was attributable to an increase in the amplitude of an LCR-induced NCX current ($0.71$ pA versus $0.27$ pA; Figure 6B, inset). This increase, in turn, resulted from an increase in LCR spatial size (more NCX within each fragment) and a larger LCR amplitude. The simulated changes in DD noise and nonlinear DD amplitude for the chronotropic interventions were consistent with our experimental findings (Figure 7C and 7D), thus predicting the importance of the nonlinear DD amplitude (originating from LCRs) with respect to beating rate regulation.

We specifically examined the mechanistic basis of the fluctuations of the exponentially rising DD phase by varying the number of LCRs in the model. It turned out that the larger LCR numbers resulted in greater nonlinear DD fluctuations and NDDC amplitudes (supplemental Figure I), thus providing direct evidence for our concept.

Finally, we observed experimentally the cycle length fluctuations of approximately $\pm 5\%$ of the mean value in a typical isolated SANC, and these are predicted, in part, by the model with LCR parameters of the basal state (Figure 7E). The fluctuations occur because of a great impact of stochastic ensemble of individual LCR/$I_{NCX}$ events on DD of each cycle.

**Discussion**

The present study, using a combination of experimental and numerical approaches, characterized the role of LCR-activated NCX current fluctuations to $V_m$ fluctuations during the DD, and demonstrated how LCR occurrence was linked to specific changes in the fine structure of the DD and how the DD fine structure controls the SANC firing rate.

The DD is characterized by its start, the MDP, slope, and its end, ie, the take-off potential of the next AP. The DD slope is an important characteristic of DD because it provides an estimate of the rate at which the membrane potential moves to AP threshold. Unfortunately, a uniform method to calculate the DD slope has not been applied in prior studies. In some studies, the mean DD slope was characterized during the first 50 to 200 ms, or the first two-thirds of the DD excluding the last, curved DD part from analysis. These discrep-
ancies could be an additional source of differences among the DD slope values obtained in the literature. For most SANCs, the DD slope gradually increases from MDP to very high values near AP threshold. In contrast, in subsidiary pacemaker cells and in atrial fibers during abnormal automaticity, DD exhibits 2 phases: an initial steeper slope followed by a more gradual slope. We observed a similar plateauing of DD in isolated rabbit SANCs in the presence of negative chronotropic interventions, when their spontaneous beating rate was reduced to that of subsidiary or abnormal pacemakers.

Based on perspectives from our previous studies demonstrating that LCRs occur during the later part of the DD and that, in most rabbit SANCs, the approximate last third of DD rises exponentially, we fit the DD by the sum of a linear function of time and an exponential term (see Figure 1A and the online data supplement). We discovered that NCX-mediated currents that contribute to DD noise, and how the ensemble of these currents affects the onset and amplitude of the NDDC. Our results also demonstrate that changes in the DD fine structure are linked to changes in the spontaneous firing rate. We observed that all negative chronotropic interventions, which, in prior studies, have been shown to suppress LCRs during DD, ie, ryanodine, buffering of intracellular $\text{Ca}^{2+}$ by BAPTA, or inhibition of $\text{Ca}^{2+}$ influx and SR $\text{Ca}^{2+}$ loading by nifedipine, delay the onset of the NDDC, reduce the amplitude of beat-to-beat fluctuations about its mean, and reduce its amplitude. In contrast, $\beta$-adrenergic stimulation increases the number, amplitude, and size of LCRs and effects their earlier occurrence. This, in turn, shifts the onset of the NDDC to an earlier time following the prior AP (supplemental Table II), enhances the amplitude of DD fluctuations, and increases the amplitude of the NDDC. Thus, SANC chronotropic state is controlled by the timing and abundance (including synchronicity) of LCR emergence after the previous AP. Because LCR emergence critically depends on SR $\text{Ca}^{2+}$ load, which, in turn, depends on $\text{Ca}^{2+}$ influx via $I_{\text{CaL}}$ and pumping $\text{Ca}^{2+}$ into SR by SERCA, the SANC operational paradigm involves a tight cooperation of both

**NDDC Controls SANC Chronotropic State**

Our experimental results and model simulations predict how the fine structure of the DD is affected by LCR-initiated NCX-mediated currents that contribute to DD noise, and how the ensemble of these currents affects the onset and amplitude of the NDDC. We also observed that LCRs contribute to the DD noise, which in turn affects the spontaneous firing rate. We found that all negative chronotropic interventions, which in prior studies have been shown to suppress LCRs, delay the onset of the NDDC, reduce the amplitude of beat-to-beat fluctuations about its mean, and reduce its amplitude. In contrast, $\beta$-adrenergic stimulation increases the number, amplitude, and size of LCRs and affects their earlier occurrence. This, in turn, shifts the onset of the NDDC to an earlier time following the prior AP (supplemental Table II), enhances the amplitude of DD fluctuations, and increases the amplitude of the NDDC. Thus, SANC chronotropic state is controlled by the timing and abundance (including synchronicity) of LCR emergence after the previous AP. Because LCR emergence critically depends on SR $\text{Ca}^{2+}$ load, which, in turn, depends on $\text{Ca}^{2+}$ influx via $I_{\text{CaL}}$ and pumping $\text{Ca}^{2+}$ into SR by SERCA, the SANC operational paradigm involves a tight cooperation of both

![Figure 6. Simulated effects of chronotropic interventions on $I_{\text{NCX}}$ and DD noise.](http://circres.ahajournals.org/)

**Figure 6.** Simulated effects of chronotropic interventions on $I_{\text{NCX}}$ and DD noise. A, Families of simulated local subspace $\text{Ca}^{2+}$ ($\text{Ca}_{\text{sub}},$ first 10 fragments), total NCX current ($I_{\text{NCX}}$), and $V_m$ under chronotropic interventions produced by ISO and ryanodine (no LCRs) (for simulation parameters, see the online data supplement). The corresponding beating rates are shown in beats per minute (bpm). B, Overlapped representative cycles from A showing changes in cycle length and diastolic NCX current amplitude and fluctuations. Inset depicts fluctuating NCX currents at the initiation of the nonlinear DD phase (boxed). $\Delta$ reflects initial changes in the NCX current related to individual LCRs.
membrane and internal Ca\(^{2+}\) cycling proteins and can be schematically presented by 2 interacting loops of membrane channel function cycling and Ca\(^{2+}\) cycling (Figure 4C). Also, an almost synchronous emergence of LCRs (resulting in the powerful NDCC) during the late DD phase requires resetting of the phases of individual LCRs. This is achieved by \(I\text{Ca}_L\) initiation of CICR and depletion of the SR Ca\(^{2+}\) load. The regulation of SANC chronotropic state via LCR interaction with the membrane ion channel cycling thus has multiple control points, some of which are affected by the perturbations used in the present study. All perturbations that affect cell or SR Ca\(^{2+}\) loading and SR Ca\(^{2+}\) release affect spontaneous RyR activation and cause changes in the LCR characteristics. Changes in spontaneous SR Ca\(^{2+}\) release during the later DD caused by nifedipine or ISO (via their affect on Ca\(^{2+}\) influx and thus Ca\(^{2+}\) available for SR Ca\(^{2+}\) pumping) are distinct from their effect on the trigger function of \(I\text{Ca}_L\) on CICR. Thus, changes in the beat-to-beat fluctuations in the \(V_m\) caused by nifedipine or ISO during the later DD report the chronotropic state, at least in part, because of their effect on LCRs. ISO, in addition, may alter LCRs via its effect on RyR phosphorylation and phospholamban\(^8\) and indirectly on L-type Ca\(^{2+}\) channels, supplying more Ca\(^{2+}\) for SR Ca\(^{2+}\) loading. ISO also enhances inwardly directed \(I\text{Ca}_L\) and other ion channels and thus may directly accelerate SANC cycle (ie, via its effect on the external loop in Figure 4C). However, our previous experiments\(^6\) and computer simulations\(^8\) indicated that the chronotropic effect of protein kinase A-dependent modulation of ion channels only (ie, without enhancement of intracellular Ca\(^{2+}\) cycling proteins) is markedly dampened.

Because the variations in timing of the onset of the NDDC or of its amplitude reflect variations in the timing and amplitude of underlying LCR-activated NCX currents, and because direct experimental evidence and numerical simulations confirm that the timing and amplitude of LCRs are tightly linked to the
spontaneous SANC firing rate,7,9 we reasoned that the LCR-induced changes in the DD fine structure ought to predict changes in the firing rate. Indeed, maneuvers that affect LCR characteristics effected changes in the fine structure of the DD that were accompanied by changes in the spontaneous firing rate, and both were highly correlated (Figure 4B). Our numerical model simulations of pacemaker currents, membrane potential, and beating rates (Figures 6 and 7) support this interpretation of the experimental correlations in Figures 3 and 4. The strong correlations between changes in the beating rate and changes in the onset of the nonlinear DD (supplemental Table II), or changes in DD fluctuations or amplitude of the later NDDC, demonstrated by the present results also further support the idea developed above, ie, that depolarizations induced by stochastic LCRs driving the NCX are a crucial component of nonlinear phase of DD in rabbit SANCs.\(^5\) (Figure 4C).

**Computer Modeling Provides a Mechanistic Insight Into NDDC and Rate Control**

Our primary SANC model, featuring stochastic LCR characteristics (see online data supplement for detail), shows that an individual LCR produces an inward current of 0.27 pA that generates a 0.17 mV DD disturbance (Figure 5). Despite the fact that the NCX current fluctuations cannot be measured experimentally during spontaneous beating, the model simulation is validated (1) by similarity of the timing and amplitude of DD noise arising from the superposition of membrane responses to individual LCRs in simulations and that observed experimentally (compare Figure 1 with Figure 7) and (2) the simulated beat-to-beat cycle length fluctuations caused by stochastic behavior of LCRs predict (at least in part) those observed experimentally (Figure 7E). To our knowledge, the latter is a novel finding and merits further study, especially as it relates to stability of pacemaker function\(^24\) (together with other known factors influencing the SANC cycle length variations such as stochastic opening and closing of individual ion channels\(^25\)).

By grading the LCR number, the model has directly tested the mechanistic basis for the exponential rising DD phase. An increase in LCR number resulted in respective increases in the NDDC amplitude and the mean DD fluctuation (supplemental Figure 1). The model thus supports the hypothesis that the fluctuating nonlinear late phase observed experimentally is caused by current fluctuations elicited by LCRs (Figure 6B, insets). We interpret these results to indicate that the superimposition/synchronization of nonlinear DD responses to individual LCRs provides a mechanism by which LCRs change the DD time course from a linear to nonlinear fashion, thus modulating the timing to reach the DD take-off-potential (Figure 4C). Interestingly, this novel regulatory mechanism does not require a highly developed SR or abundant RyR expression,\(^26\) because the RyR \(\text{Ca}^{2+}\) release signaling requires that only those RyRs that are close to the plasma membrane produce LCRs; the initial signaling is subsequently greatly amplified by the (voltage-gated) ion channel mechanisms.

**The Initial Linear DD Component**

Our experiments also showed that the slope of the initial linear DD component decreased significantly under BAPTA, and increased under ISO (supplemental Table II), suggesting an additional important role of \(I_t\), delayed rectifier potassium, and other currents during the initial part of diastolic depolarization. Although a recent study suggests that \(I_t\), passing only Na\(^+\) and K\(^+\),\(^27\) is implicated in cAMP modulation of SANC firing rate, it would appear to contribute mostly to the linear DD component, which, under control conditions, might be considered as its \(\text{Ca}^{2+}\)-independent part, because most (>80%) LCRs occur during the later exponential DD phase, ie, during the last third of the DD\(^5\) (Figure 2B). Other \(\text{Ca}^{2+}\)-independent mechanisms, eg, modulation of delayed rectifier potassium current, may also affect a beating rate via regulating MDP and the slope of the linear DD component. Although \(\text{Cs}^+\), used in our study to block \(I_t\), is not specific to \(I_f\) blocker, 2 mmol/L \(\text{Cs}^+\) produces the same effect on the AP parameters and the spontaneous beating rate as more specific \(I_f\) blockers, UL-FS-49 and ZD-7288.\(^16\)

Several potassium currents in the SAN could be affected by \(\text{Cs}^+\). However, \(I_{Kr}\) is absent in the rabbit SA node; \(I_{Kr}\) is present only in small amount of cells in the periphery; and \(I_K\) plays the most prominent role in the rabbit SA node. However, effect of \(\text{Cs}^+\) on the \(I_{Kr}\) does not offset its effect on \(I_f\), because deactivation of \(I_{Kr}\) is a major determinant of the linear part of the DD,\(^7\) and its suppression by \(\text{Cs}^+\) would induce an additional decrease in the slope of diastolic depolarization and, as a result, in the spontaneous beating rate. The very modest effect of \(\text{Cs}^+\) on the DD slope and spontaneous beating rate observed in our experiments suggests \(I_{Kr}\) is not significantly affected by \(\text{Cs}^+\).

In summary, spontaneous, stochastic but roughly periodic LCRs occurring during the later NDDC activate local NCX currents that produce depolarizations of sufficient magnitude to produce membrane noise, detectable as beat-to-beat fluctuations in membrane potential during this part of the DD. The timing and magnitude of these \(\text{Ca}^{2+}\)-dependent DD membrane potential fluctuations reflects the timing and magnitude of the underlying LCR. Thus, the status of LCR characteristics are reflected in the fine structure of the DD and via its control of the nonlinear DD characteristics, LCR control the SANC firing rate. This influence of intracellular \(\text{Ca}^{2+}\) release on SANC automaticity has a counterpart in other cardiac cell types (see online data supplement and\(^28\)).

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**Disclosures**

None.

**References**


Online Supplement for the manuscript by Bogdanov et al.

Analysis of DD components and computation of DD variability

The interval beginning with the maximum diastolic depolarization and ending when membrane potential reached -20 mV was utilized for DD calculations. The membrane potential of -20 mV was chosen as the end of the DD because the rate of depolarization reached about of 50% of its maximal value at this potential and this was considered as the start of AP upstroke. The mean DD of a given state was determined by averaging local means of several ($n=20-50$) successive 16-sec long records of spontaneous APs.

The mean DD slope (MDDS) during the first two-thirds of the DD$^1$ was calculated excluding the last, curved DD part from analysis. Then, the amplitude of DD non-linear component (NDDC) was derived as follows: $DD(t) = NDDC \times \exp(-(t_0-t)/\text{tau}) + \text{MDDS} \times t + C$, using custom-written programs based on the IDL 5.2 programming language (Research System, Boulder, USA), where $DD(t)$ is the pacemaker potential at time $t$, $t_0$ is the time when $DD(t)$ reaches -20 mV, $\text{tau}$ is a time constant, and $C$ is an offset constant. The AP take-off (TOP) potential was calculated as the membrane potential estimated by extrapolating the initial linear part of DD to the moment when the AP maximal $\text{dv/dt}$ was reached. The amplitude of linear component was calculated as the difference between TOP and MDP (see Fig.1 of the main text). This method of determining DD components is more accurate than previous ones$^{2-5}$, and in most cases the $R^2$ for the fit was close to 0.99. As mentioned above, we assume that DD ends when it reaches -20 mV, corresponding to a moment when the first derivative of membrane
potential reaches a level of about 50% of its maximal value. We performed similar analysis assuming that DD ends at -30 or -10 mV. As expected, the amplitude of non-linear component was higher when -10 mV was chosen, and lower for -30 mV. In both cases, however, the sensitivity of non-linear component to perturbations affecting LCRs (see below) was similar to that when -20 mV was chosen as cut-off potential.

The beat-to-beat DD fluctuations in the 100-ms segment preceding AP upstroke was measured as the residual variance in each beat after the group mean DD had been subtracted. Specifically, the DD records for a given cell under a given condition were processed as follows: the mean DD ($DD_m$) and variance estimates were calculated from the group of $n$ records by evaluating $DD_m = (\text{SUM of } DD_i)/n$, and variance $= (\text{SUM of } \delta DD_i)/(n-1)$, where $DD_i$ is the $i^{th}$ DD record and $\delta DD_i$ is the square of deviation of $DD_i$ sample from its group mean value. The calculations were done off-line so that the experimenter could reject those groups of records having noise spikes or unusually large variance values. The mean value of DD fluctuations for a given cell was calculated as the average of absolute fluctuations of each DD sample from its group mean value over a 50-ms interval preceding the next AP upstroke. This 50-ms interval was chosen because the DD fluctuations increased with time following a prior AP and reached a maximum at 20-40 ms before the subsequent AP upstroke. Neither DD variance nor mean value of DD fluctuations changed significantly when the end of DD was considered to be -10 or -30 mV rather than -20 mV.

**Numerical SANC model featuring stochastic LCRs**
**Model assumptions**

To simulate the membrane potential noise produced by local NCX currents initiated by individual LCRs, we employed a modification of the most recent version of a SANC numerical model which embraced experimentally measured individual LCR characteristics (Online Supplement in \(^6\)). In short, we assigned sets of “local” variables describing ion dynamics within each individual LCR and its adjacent proximity. Particularly, we considered that each LCR interacts only with an adjacent membrane fragment, limited to an average effective LCR area \((S_{\text{LCR}})\).

**Introduction of the local variables**

We introduced \(N_{\text{max}}\), a maximum number of LCR due to membrane surface restrictions, i.e. \(N_{\text{max}} = S_{\text{cell}}/S_{\text{LCR}} \sim 177\) (an estimate is given below). The subspace, junctional SR, network SR, and myoplasm are then virtually divided into the \(N_{\text{max}}\) fragments, with each fragment reflecting ion dynamics in the proximity of an LCR. To avoid unnecessary complexity, we describe only average values within each fragment and neglect interactions between fragments. Our local variables describe the ion homeostasis in each \(i\)-th fragment \((i \in [1,N_{\text{max}}])\) (for example \(\text{Ca}_{\text{sub},i}, \text{Ca}_{\text{rel},i}, \text{I}_{\text{NaCa},i}, \text{I}_{\text{CaL},i}\), etc., see a complete list in online Table 1). The relations between local variables are basically the same as defined originally by Kurata et al.\(^7\) for the whole cell, with the addition of the spontaneous \(\text{Ca}^{2+}\) release flux introduced by Maltsev et al.\(^8\) and redefined below. Other variables are considered as global and thus being equal in any fragment, e.g. \(\text{K}^{+}\) concentration, membrane potential, etc. Ion membrane currents were calculated for each fragment and the respective global currents were calculated as a simple summation, for
example,

\[ I_{Ca\ell} = \sum_{i=1}^{N} I_{Ca\ell,i} \]

\[ I_{Na\ell} = \sum_{i=1}^{N} I_{Na\ell,i} \]

In turn, the sum of global membrane currents, \( I_{total} \), determined the \( \frac{dV}{dt} \) used for the model integration:

\[ \frac{dV}{dt} = - \frac{I_{total}}{C_m} \]

**Numeric approximation of individual LCRs**

The \( \text{Ca}^{2+} \) flux in each junctional SR fragment has instantaneous and spontaneous (time-dependent) components, \( j_{\text{inst},i} \) and \( j_{\text{spont},i} \), respectively:

\[ j_{\text{inst},i} = P_{\text{rel,inst}} \cdot (C_{a\ell,i} - C_{a\ell,\text{sub},i})/[1 + (K_{\text{rel}}/C_{a\ell,\text{sub},i})^2] \]

\[ j_{\text{spont},i} = P_{\text{phase},i}(t) \cdot P_{\text{rel,spont}} \cdot (C_{a\ell,i} - C_{a\ell,\text{sub},i}) \]

We simplified a cosine \( P_{\text{phase},i}(t) \) function used in 7 to all-or-none phasic function:

\[ P_{\text{phase},i}(t) = 1 \text{ for } t_{\text{phase},i} < t < t_{\text{phase},i} + t_{\text{width}} \]

\[ P_{\text{phase},i}(t) = 0 \text{ for } t < t_{\text{phase},i} \text{ and } t > t_{\text{phase},i} + t_{\text{width}} \]

where \( t_{\text{width}} \) is an LCR duration (~ 48.53 ms). Each LCR phase, \( t_{\text{phase},i} \), is assigned in each duty cycle by the computer program which generates random numbers with Gaussian distribution about the mean phase, \( t_{\text{phase}} \), and a standard deviation, SD, reflecting a synchronicity of LCR occurrence. Each cycle start was caught by the computer program when the membrane voltage was rising and crossed the zero line. For simplicity we neglect detailed dynamics of individual RyR recruitment during LCR, so that the above \( P_{\text{phase},i}(t) \) function assumes a simultaneous opening of a fixed number of RyRs in the
respective $i$-th junctional SR fragment at the time $t_{\text{phase},i}$ and all RyRs stay open for an effective time $t_{\text{width}}$.

**Relationship between experimental and model parameters**

The relations between experimental and model parameters were established as follows.

*Estimation of the LCR effective area and LCR maximum number*

Our most recent experimental measurement of LCR half width (a mean value) is $L_{\text{LCR}} = 4.8 \, \mu\text{m}$. The effective LCR area, $S_{\text{LCR}} = \pi \cdot 4.8^2 / 4 \approx 18.1 \, \mu\text{m}^2$, and the electrical capacitance of the membrane fragment effectively interacting with an individual LCR is $C_1 = 18.1 \, \mu\text{m}^2 \cdot 0.01 \, \text{pF}/\mu\text{m}^2 = 0.181 \, \text{pF}$. The estimate of a maximum number of LCR in a 32 pF cell (i.e. LCRs interact with whole cell surface) is $N_{\text{max}} = 32 / 0.181 \approx 177$. Note that under ISO stimulation $L_{\text{LCR}}$ was larger resulting in a lesser $N_{\text{max}} \approx 103$.

*The number of LCRs per cycle*

Although the number of virtual cell fragments was set 177, we allowed developing LCR only in some of them with respect to a frequency of LCR occurrence. We measured an average number of LCRs per cycle ($n_{\text{LCR, line}} \approx 1.24$ in control cells) that, in fact, estimates how often an LCR occurs in any part of the subspace within a typical scanning line $L_{\text{scan}}$ of $\approx 25 \, \mu\text{m}$. Since an LCR average size ($L_{\text{LCR}}$) along the scanning line is $4.8 \, \mu\text{m}$, the fraction of the scanning line occupied with LCR can be estimated as $f = n_{\text{LCR, line}} \cdot L_{\text{LCR}} / L_{\text{scan}} = 1.24 \cdot 4.8 / 25 = 0.23808$. Since the average scanning line represents $\text{Ca}^{2+}$ signals in the total subspace, the same fraction of the subspace is expected to be populated with LCRs. Finally, the number of LCRs per cycle is estimated as
\[ N_{\text{LCR}} = N_{\text{frag}} \cdot f = 177 \cdot 0.23808 \approx 42. \] While the estimated number of LCR per cycle is about 40, in our simulations we actually explored the effects of the LCR number in a wide range from 10 to 100.

**The LCR phase**

The \( t_{\text{phase}} \) reflects initiation of the model release but experimentally, LCR periods (\( T_{\text{LCR}} \)) were measured from systolic peak to LCR peak. As the LCR peak develops in our model approximately 20 ms after its initiation, we assumed that \( t_{\text{phase}} = <T_{\text{LCR}}> - 20 \text{ ms} \).

**The LCR amplitude**

Spontaneous Ca\(^{2+}\) release rate \( P_{\text{rel,spont}} \) and SR Ca\(^{2+}\) uptake rate \( P_{\text{up}} \) could not be directly assessed and thus were adjusted to satisfy the simulated LCR amplitude to match that observed experimentally. An estimate of 626 nM for an LCR amplitude is derived from a typical value for the LCR peak \( F/F_0 \) value of ~2.2 using a formula \(^{10}\)

\[
[\text{Ca}^{2+}] = K_d \cdot \frac{(F/F_0)}{(K_d/[\text{Ca}^{2+}]_{\text{rest}} + 1 - F/F_0)},
\]

where \( K_d = 864 \text{ nM} \) \(^{11}\) and \( [\text{Ca}^{2+}]_{\text{rest}} \) of about 206 nM \(^8\).

**The LCR duration**

The experimentally assessed estimate for LCR duration is the full duration at half maximum amplitude, \( \text{FDHM} = 48.53 \text{ ms} \). We assigned this estimate to the parameter \( t_{\text{width}} \) determining the release duration in the model (Equation 2).

**The synchronicity of LCR occurrence**

The LCR synchronicity is determined by the SD of the \( t_{\text{phase,i}} \) random number generator. To match experimentally observed LCRs’ occurrence spread within 10\% of the total cycle length \(^8,9\), we assigned a value of 32 ms to SD.
Computer simulations of the effects of chronotropic interventions

We used the following sets of the model parameters to simulate effects of chronotropic interventions.

**CONTROL:** $L_{LCR} = 4.8 \, \mu m; \ t_{width} = 48.53 \, ms; \ P_{rel, inst} = 0.5 \, ms^{-1}; \ t_{phase} = 380 \, ms; \ number \ LCR \ per \ cycle, \ N_{LCR} = 65$ (we also varied it from 10 to 100, see below); subspace width $L_{sub} = 0.04 \, \mu m$; the rate constant for Ca$^{2+}$ uptake by the Ca$^{2+}$ pump in the network SR, $P_{up} = 0.008 \, M/s$; time constant of Ca$^{2+}$ diffusion from the subspace $\tau_{dif, Ca} = 0.07 \, ms^{-1}$; plasma membrane conductances (in nS/pF) $g_{CaL} = 0.203, \ g_{bNa} = 0.00162, \ g_{CaT} = 0.2748, \ g_h = 0.225$; $g_s = 0.003, \ g_{Kr} = 0.0422603, \ g_{Ks} = 0.01295, \ g_{to} = 0.09, \ g_{sus} = 0.01, \ g_{KACa} = 0.00109811, \ g_{hNa} = 0.0006, \ g_{Na} = 0; \ I_{NaKmax} = 2.25 \, pA/pF; \ k_{NaCa} = 125 \, pF^{-1}$.

**ISO:** $L_{LCR} = 6.3 \, \mu m; \ t_{width} = 60 \, ms; \ t_{phase} = 270 \, ms; \ P_{up} = 0.013 \, M/s; \ g_{CaL} = 0.406 \, nS/pF; \ g_{h} = 0.45 \, nS/pF; \ g_{Kr} = 0.0845206 \, nS/pF$. Other parameters were the same as in control.

**RYANODINE** (no LCRs): $N_{LCR} = 0$; other parameters were the same as in control.
We also simulated the effect of graded LCR numbers on the fluctuating nonlinear DD component. The results of these simulations are shown in online Figure I.

**Online Figure I.** Model simulations directly test the mechanism of the fluctuating nonlinear DD component as originated from multiple LCRs occurring under the cell surface membrane. An increase in the number of LCRs results in increases of the NDD fluctuations (panel A) and the amplitude of NDD component (Panel B). “CONTROL” parameter set was used in simulations with varying number of LCRs ($N_{\text{LCR}}$).

**Spontaneous SR Ca$^{2+}$ oscillations generate automaticity in various cardiac cell types other than sinoatrial nodal pacemaker cells**

Spontaneous, RyR SR-generated oscillations of intracellular Ca$^{2+}$ have previously been indirectly detected as force oscillations in skinned cardiac cells$^{12}$ or spontaneous beating in electrochemically shunted cells$^{13}$ and by laser light scattering technique or recordings of voltage, or current, myofilament motion or tension in unstimulated, multicellular
cardiac muscle and Purkinje fibers\textsuperscript{12,14-22} (see\textsuperscript{23} for review). With the advent of intracellular Ca\textsuperscript{2+} probes (see\textsuperscript{23-24} for review) the underlying Ca\textsuperscript{2+} oscillations were subsequently directly demonstrated. Unlike SANC, these spontaneous Ca\textsuperscript{2+} oscillations in ventricular cells require experimental cell Ca\textsuperscript{2+} loading and originate locally within cells and while stochastic, they are roughly periodic and their periodicity (<0.1 Hz to about 7 Hz) varies with cell and SR Ca\textsuperscript{2+} loading. Further, during electrical stimulation, action potential triggered and spontaneous Ca\textsuperscript{2+} release are interactive with respect to their magnitude and occurrence (see\textsuperscript{23} for review). In ventricular, atrial, and Purkinje fiber cells, spontaneous SR Ca\textsuperscript{2+} oscillations produce delayed after-depolarizations\textsuperscript{25} driven by a calcium-activated transient inward current which may initiate arrhythmias\textsuperscript{26-27}. Spontaneous Ca\textsuperscript{2+} release of this sort leads to spontaneous depolarization of ventricular myocytes and is a cause of “abnormal automaticity” of these cells\textsuperscript{28}. The present study and previous ones\textsuperscript{29-31} show that SR Ca\textsuperscript{2+} release via RyR plays an important role in normal automaticity of rabbit SANCs modulating their spontaneous beating rate over a wide (1-4 Hz) range.

**ONLINE SUPPLEMENT REFERENCES:**


2. Opthof T, de Jonge B, Mackaay AJ, Bleeker WK, Masson-Pevet M, Jongsma HJ, Bouman LN. Functional and morphological organization of the guinea-pig sinoatrial node compared with the rabbit sinoatrial node. *J Mol Cell Cardiol*. 1985;17:549-564.


ryanodine receptor Ca\textsuperscript{2+} releases during diastolic depolarization of sinoatrial pacemaker
10. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying
11. Merritt JE, McCarthy SA, Davies MP, Moores KE. Use of fluo-3 to measure
cytosolic Ca\textsuperscript{2+} in platelets and neutrophils. Loading cells with the dye, calibration of
traces, measurements in the presence of plasma, and buffering of cytosolic Ca\textsuperscript{2+}. *Biochem
12. Fabiato A, Fabiato F. Myofilament-generated tension oscillations during partial
calcium activation and activation dependence of the sarcomere length-tension relation of
13. Chiesi M, Ho MM, Inesi G, Somlyo AV, Somlyo AP. Primary role of sarcoplasmic
reticulum in phasic contractile activation of cardiac myocytes with shunted myolemma. *J
*Pflugers Arch.* 1975;358:11-25.
15. Lappe DL, Lakatta EG. Intensity fluctuation spectroscopy monitors contractile
16. Kass RS, Tsien RW. Fluctuations in membrane current driven by intracellular
17. Stern M, Kort A, Bhatnagar G, Lakatta EG. Scattered-Light Intensity Fluctuations in
Diastolic Rat Cardiac Muscle Caused by Spontaneous Ca\textsuperscript{2+} - dependent Cellular Mechanical Oscillations. J Gen Physiol. 1983;82:119-153.


### Online Table I. List of “local” variables determining LCR in the $i$-th subspace fragment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{CaL,i}$</td>
<td>L-type Ca$^{2+}$ current</td>
</tr>
<tr>
<td>$f_{Ca,i}$</td>
<td>Ca$^{2+}$-dependent inactivation gating variable for $I_{CaL,i}$</td>
</tr>
<tr>
<td>$I_{NaCa,i}$</td>
<td>NCX current</td>
</tr>
<tr>
<td>$I_{bCa,i}$</td>
<td>Ca$^{2+}$-background current</td>
</tr>
<tr>
<td>$Ca_{sub,i}$</td>
<td>Ca$^{2+}$ concentration in the subspace</td>
</tr>
<tr>
<td>$Ca_{i,i}$</td>
<td>Myoplasmic Ca$^{2+}$ concentration</td>
</tr>
<tr>
<td>$Ca_{rel,i}$</td>
<td>Ca$^{2+}$ concentration in the junctional SR</td>
</tr>
<tr>
<td>$Ca_{up,i}$</td>
<td>Ca$^{2+}$ concentration in the network SR</td>
</tr>
<tr>
<td>$t_{phase,i}$</td>
<td>LCR phase</td>
</tr>
<tr>
<td>$j_{spont,i}$</td>
<td>Spontaneous Ca$^{2+}$ flux from junctional SR to subspace</td>
</tr>
<tr>
<td>$j_{inst,i}$</td>
<td>Instantaneous Ca$^{2+}$ flux from junctional SR to subspace</td>
</tr>
<tr>
<td>$f_{CML,i}$</td>
<td>Fractional occupancy of calmodulin by Ca$^{2+}$ in myoplasm</td>
</tr>
<tr>
<td>$f_{CMS,i}$</td>
<td>Fractional occupancy of calmodulin by Ca$^{2+}$ in subspace</td>
</tr>
<tr>
<td>$f_{CQ,i}$</td>
<td>Fractional occupancy of calsequestrin by Ca$^{2+}$</td>
</tr>
<tr>
<td>$f_{TC,i}$</td>
<td>Fractional occupancy of the troponin-Ca site by Ca$^{2+}$</td>
</tr>
<tr>
<td>$f_{TMC,i}$</td>
<td>Fractional occupancy of the troponin-Mg site by Ca$^{2+}$</td>
</tr>
<tr>
<td>$f_{TMM,i}$</td>
<td>Fractional occupancy of the troponin-Mg site by Mg$^{2+}$</td>
</tr>
<tr>
<td>$Q_{ci,i}$</td>
<td>Fractional charge movement during intracellular Ca$^{2+}$ occlusion reaction of $I_{NaCa,i}$ transporter</td>
</tr>
<tr>
<td>$Q_{co,i}$</td>
<td>Fractional charge movement during extracellular Ca$^{2+}$ occlusion reaction of $I_{NaCa,i}$ transporter</td>
</tr>
<tr>
<td>$Q_{n,i}$</td>
<td>Fractional charge movement during Na$^+$ occlusion reactions of $I_{NaCa,i}$ transporter</td>
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### Online Table II. Characteristics of rabbit SANC AP and DD in control and in response to chronotropic interventions

<table>
<thead>
<tr>
<th></th>
<th>lumped control</th>
<th>control</th>
<th>ryanodine</th>
<th>control</th>
<th>BAPTA</th>
<th>control</th>
<th>Nifedipine</th>
<th>control</th>
<th>ISO</th>
<th>control</th>
<th>Cs⁺</th>
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<tr>
<td>SANC number</td>
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<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
<td>12</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Dose, μmol/L</td>
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<td>5</td>
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<td>1</td>
<td>1</td>
<td>2000</td>
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<tr>
<td>Beating rate, bpm</td>
<td>172±5</td>
<td>168±7</td>
<td>105±4 *</td>
<td>149±13</td>
<td>69±10*</td>
<td>182±10</td>
<td>125±6*</td>
<td>165±8</td>
<td>203±5*</td>
<td>206±6</td>
<td>195±6*</td>
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<td>MDP, mV</td>
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<td>-57±2</td>
<td>-50±5</td>
<td>-44±2</td>
<td>-57±2</td>
<td>-58±2</td>
<td>-56±1</td>
<td>-59±1*</td>
<td>-58±2</td>
<td>-57±1</td>
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<td>OS, mV</td>
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<td>29±5</td>
<td>41±4*</td>
<td>34±2</td>
<td>38±1</td>
<td>41±2</td>
<td>40±4</td>
<td>36±2</td>
<td>35±2</td>
<td>36±2</td>
<td>40±2*</td>
</tr>
<tr>
<td>Max dV/dt, V/s</td>
<td>13±1</td>
<td>16±6</td>
<td>20±5*</td>
<td>7±2</td>
<td>6±1</td>
<td>13±3</td>
<td>17±4*</td>
<td>14±2</td>
<td>18±3*</td>
<td>9±1</td>
<td>11±1</td>
</tr>
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<td>APD50, ms</td>
<td>104±4</td>
<td>99±10</td>
<td>125±11*</td>
<td>113±12</td>
<td>171±33*</td>
<td>105±5</td>
<td>98±13</td>
<td>107±9</td>
<td>100±5</td>
<td>91±7</td>
<td>95±8*</td>
</tr>
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<td>TOP, mV</td>
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<td>-47±2</td>
<td>-37±2*</td>
<td>-39±4</td>
<td>-29±3*</td>
<td>-46±1</td>
<td>-39±2*</td>
<td>-46±2</td>
<td>-50±2*</td>
<td>-48±1</td>
<td>-50±1</td>
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<tr>
<td>DD duration, ms</td>
<td>194±9</td>
<td>204±25</td>
<td>379±29*</td>
<td>214±26</td>
<td>612±131*</td>
<td>175±21</td>
<td>316±11*</td>
<td>204±14</td>
<td>147±9*</td>
<td>154±12</td>
<td>163±10</td>
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<tr>
<td>MDD slope, mV/s</td>
<td>57±4</td>
<td>66±12</td>
<td>47±4</td>
<td>60±11</td>
<td>19±3*</td>
<td>66±10</td>
<td>53±6</td>
<td>47±7</td>
<td>73±7*</td>
<td>58±3</td>
<td>57±8</td>
</tr>
<tr>
<td>NDDC amplitude, mV</td>
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<td>22±2</td>
<td>12±2*</td>
<td>18±4</td>
<td>10±1*</td>
<td>19±2</td>
<td>14±2*</td>
<td>19±1</td>
<td>23±1*</td>
<td>23±2</td>
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<td>NDDC tau, ms</td>
<td>17±1</td>
<td>17±1</td>
<td>16±2</td>
<td>22±3</td>
<td>28±2*</td>
<td>13±2</td>
<td>21±4</td>
<td>18±2</td>
<td>13±1*</td>
<td>14±1</td>
<td>15±1</td>
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<tr>
<td>NDDC delay, ms</td>
<td>145±8</td>
<td>156±27</td>
<td>347±33*</td>
<td>149±27</td>
<td>527±112*</td>
<td>140±21</td>
<td>261±12*</td>
<td>150±11</td>
<td>108±8*</td>
<td>118±8</td>
<td>121±8</td>
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</table>

Lumped control, sum of control records for all cells preceding interventions; MDP, maximum diastolic potential; OS, AP overshoot; APD50, AP duration measured at 50 % of repolarization; TOP, take-off potential; MDD slope - a mean DD slope measured during the first two-thirds of the DD; NDDC - non-linear diastolic depolarization component; NDDC tau, a time constant of exponential fitting of non-linear component; NDDC delay, an interval between a moment when MDP was reached and timing when NDDC reaches 5 % of is amplitude value; *, P<0.05 vs control.