Published in final edited form as:

J Mol Cell Cardiol. 2011 December ; 51(6): 902–905. doi:10.1016/j.yjmcc.2011.08.029.

Beat to beat Ca^{2+} -dependent regulation of sinoatrial nodal pacemaker cell rate and rhythm

Yael Yaniv^{1,*}, Victor A. Maltsev^{1,*}, Ariel L. Escobar^{2,*}, Harold A. Spurgeon¹, Bruce D. Ziman¹, Michael D. Stern¹, and Edward G. Lakatta¹

¹Laboratory of Cardiovascular Science, Gerontology Research Center, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA

²School of Engineering, Univ. of California-Merced, Merced, CA

Abstract

Introduction—Whether intracellular Ca^{2+} regulates sinoatrial node cell (SANC) action potential (AP) firing rate on a beat-to-beat basis is controversial.

Objective—To directly test the hypothesis of beat-to-beat intracellular Ca^{2+} regulation of the rate and rhythm of SANC.

Methods and results—We loaded single isolated SANC with a caged Ca^{2+} buffer, NP-EGTA, and simultaneously recorded membrane potential and intracellular Ca^{2+} . Prior to introduction of the caged Ca^{2+} buffer, spontaneous local Ca^{2+} releases (LCRs) during diastolic depolarization (DD) were tightly coupled to rhythmic APs ($r^2=0.9$). The buffer markedly prolonged the decay time (T_{50}) and moderately reduced the amplitude of the AP-induced Ca^{2+} transient and partially depleted the SR load, suppressed spontaneous diastolic LCRs and uncoupled them from AP generation, and caused AP firing to become markedly slower and dysrhythmic. When Ca^{2+} was acutely released from the caged compound by flash photolysis, intracellular Ca^{2+} dynamics were acutely restored and rhythmic APs resumed immediately at a normal rate. After a few rhythmic cycles, however, these effects of the flash waned as interference with Ca^{2+} dynamics by the caged buffer was reestablished.

Conclusions—Our results directly support the hypothesis that intracellular Ca^{2+} regulates normal SANC automaticity on a beat-to-beat basis.

Keywords

pacemaker cell automaticity; Ca^{2+} cycling; pacemaker Ca^{2+} clock; Ca^{2+} -excitation contraction coupling; arrhythmia

Address for correspondence: Edward G. Lakatta, Laboratory of Cardiovascular Science, Gerontology Research Center, Intramural Research Program, National Institute on Aging, NIH, 5600 Nathan Shock Dr., Baltimore, MD 21224-6825 USA.

LakattaE@mail.nih.gov.

*These authors contributed equally to this work

Disclosures

None declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

A novel hypothesis for the basis of sinoatrial nodal cell (SANC) normal automaticity, supported by experiments in numerous species generated by many labs (cf [1] for review) postulates that surface membrane electrogenic proteins of SANC, functioning as a voltage oscillator (M clock), and intracellular proteins, generating rhythmic Ca^{2+} oscillations (Ca^{2+} clock), interact as a system of symmetrically entrained, inter-dependent oscillators.

The hypothesis embraces several key interactions resulting in clock entrainment. Following an action potential (AP), SERCA pumps Ca^{2+} from the cytosol into a sarcoplasmic reticulum (SR) that is partially depleted of Ca^{2+} , due to periodic Ca^{2+} release triggered by the AP. Shortly after the maximum diastolic potential, when sufficient Ca^{2+} has been pumped to achieve a threshold SR Ca^{2+} load required for spontaneous ryanodine receptor (RyR) activation, RyRs begin to generate spontaneous local Ca^{2+} releases (LCRs) beneath the cell surface membrane. LCRs grow in number and size during the diastolic depolarization (DD). Their contribution to the DD, via activation of Na^+ - Ca^{2+} exchange (NCX) current, also grows exponentially, and this is a major mechanism that drives the DD to threshold for activation of L-type current, which initiates the next AP. The AP, in turn, by (1) inducing Ca^{2+} release from SR, synchronizes the SR in a relatively Ca^{2+} depleted state and (2) by effecting Ca^{2+} influx, restores SR Ca^{2+} lost from the cell via NCX. Thus, during each cycle the AP “resets” and “rewinds” the SR function within the coupled-clock system.

The coupled-clock system pacemaker hypothesis challenges a long-standing pacemaker research community tenet that the membrane clock is the sole or dominant mechanism for normal automaticity of SANC [2, 3]. Acute photolysis of intracellular caged Ca^{2+} compound has provided novel mechanistic insights into beat-to-beat Ca^{2+} cycling in ventricular myocytes [4–7]. Experiments to determine whether impaired SR Ca^{2+} cycling dynamics by an intracellular caged Ca^{2+} buffer, or an acute increase in intracellular Ca^{2+} via photolysis of the caged- Ca^{2+} buffer regulates SANC AP firing, would be a stringent test of the hypothesis that a coupled clock system regulates normal automaticity specifically on a beat-to-beat basis.

Methods

We loaded NP-EGTA, a caged Ca^{2+} buffer, into single, isolated rabbit SANC and simultaneously recorded membrane potential via a perforated patch, and imaged Ca^{2+} by confocal microscopy to determine the effects on AP firing in SANC, of: (1) Ca^{2+} buffering by the caged EGTA; and (2) the acute increase in intracellular Ca^{2+} induced by flash photolysis of the Ca^{2+} buffer. A detailed expanded methods description is available in the Online Data Supplement.

Results and Discussion

Prior to Ca^{2+} buffer loading, SANC generate spontaneous rhythmic diastolic LCRs that are highly coupled to the subsequent AP occurrence (Fig. 1A). In response to loading of the Ca^{2+} buffer, the kinetics of Ca^{2+} removal from the cytosol are markedly slowed (Fig. 1B and Table S1). The integral of Ca^{2+} release in the form of LCRs (detected by the Ca^{2+} indicator) becomes substantially reduced (Fig. 1C). The LCR period, defined as the time from the post AP-induced Ca^{2+} transient peak to LCR occurrence, becomes prolonged and dysrhythmic. As a result, LCRs become uncoupled from AP generation, which becomes markedly slowed and then dysrhythmic (Fig. 1D). Coefficient of variation (SD/mean) of AP cycle length (CL) prior to NP-EGTA loading was $7 \pm 1.5\%$ and increased at 2 min and 5 min following NP-EGTA loading to $9 \pm 2\%$ and $20 \pm 5\%$, respectively ($n=7$ cells). Note that the

LCR period closely predicted each AP CL prior to introduction of Ca^{2+} buffer ($r^2=0.9$, $p<0.01$), but not after ($r^2=0.4$) (Fig. 1D). Average data on AP induced transients, LCR characteristics and AP CL (n=7 cells) before and after NP-EGTA application are provided in the online supplement Table S1.

Figure 2 illustrates the effect of photolysis to release Ca^{2+} in a cell that had been preloaded with the caged buffer. Prior to flash photolysis, AP firing rate is slow and dysrhythmic and LCRs are small and partially uncoupled from AP generation (Fig.2A). The SR Ca^{2+} load was reduced by NP-EGTA (the amplitude of the Ca^{2+} transient induced by a rapid application of caffeine onto the cells decreased from 1.6 ± 0.1 to 1.3 ± 0.1 F/F_0 , n=9 in each group). Four 50 ms flashes were applied within a time interval of about 150 ms (black arrows in Fig. 2B). Although small in magnitude (Fig. S1), the photo-released Ca^{2+} dramatically affects SANC function. The AP-induced Ca^{2+} transient, LCR characteristics/pattern, the DD slope, and the pattern of AP firing are affected (Fig. 2C-D). The initial effect of the flashes to markedly accelerate DD and acutely reduce CL by ~50% occurs after the termination of the last flash (between beats 3 and 4 in Figure 2B and shown in a slower time base in Fig.2F) and corresponds in time to the emergence of augmented and synchronous LCRs, the ensemble of which produces substantial Late Diastolic Ca^{2+} Elevation (“LDCaE” in Fig. 2E-F) (reaching the red color of F/F_0 in the second cycle). This strongly suggests that the marked increase in DD slope, and reduction in CL induced by the flash, do not result directly from Ca^{2+} release during the flash, but rather require SR pumping of Ca^{2+} released by the flash and subsequent SR Ca^{2+} release, resulting in the emergence of large, abundant LCRs. In other words, the acute, transient, marked reduction in CL caused by the flash was likely mediated by its effects to markedly increase LCRs, which accelerate the DD via Ca^{2+} -dependent effects on sarcolemmal molecules. It cannot be directly predicted, however, to what extent the reduction in CL is attributable to augmentation in LCRs that resulted from SR Ca^{2+} cycling resulting from the flash, or to a direct effect of flash-released Ca^{2+} on sarcolemmal proteins.

The phase-plane diagram of the respective duty cycles of the Ca^{2+} signal and the membrane potential (Fig. 2G) illustrates that photo-released Ca^{2+} produces a profound change in the coupling of Ca^{2+} and M-clocks. Prior to the flash, the Ca^{2+} -membrane-voltage loop cycles counterclockwise throughout the cycle, but following the flash the loop cycles half clockwise/half counterclockwise (Fig. 2G). The flash-induced clockwise cycling at the beginning of the loop at low diastolic potentials reflects the emergence of the strong diastolic Ca^{2+} signal that accelerates the DD. Similar trends were found in nine of the SANC. After three rhythmic APs shown in Fig.2B (AP recording), the effects of the flash waned, likely due to reestablishment of altered/impaired SR Ca^{2+} cycling dynamics by Ca^{2+} by the caged buffer, and SANC AP firing rate again becomes slower and dysrhythmic on a beat-to-beat basis.

Numerous prior studies, in a variety of species have demonstrated that intracellular Ca^{2+} buffering by BAPTA-AM or EGTA-AM markedly slows the spontaneous AP firing rate, but intracellular Ca^{2+} was not measured in these cells (review [1]). However, by using caged Ca^{2+} buffer and measuring intracellular Ca^{2+} we examined not only the link between impaired Ca^{2+} cycling dynamics and disturbed AP firing affected by intracellular Ca^{2+} buffering, but also the beat-to-beat relationship between intracellular Ca^{2+} cycling and AP generation by an acute increase in intracellular Ca^{2+} accomplished by flash photolysis.

Of note, recent reports challenge the existence or symmetry of the coupled-clock system [2, 3]. Introduction of BAPTA into guinea pig SANC via acute rupture of a surface membrane patch failed (in the absence of intracellular Ca^{2+} measurements) to detect **any** acute reduction in AP firing rate [3]. The null result, which conflicts with the present and

numerous other studies (cf [1] for review) can be explained on the basis of the method to apply BAPTA via a pipette; i.e. a small, artifactual rupture-induced patch seal leak current that shifts the current balance of tiny DD currents offsetting the true BAPTA effect that would, in the absence of the artifact, suppress the DD and prolong the cycle length [8] as demonstrated by numerical modeling of the null results [8].

In summary, our results provide direct evidence to support the hypothesis that a system of an intracellular Ca^{2+} clock, coupled to a surface membrane clock [1], rather than a dominant surface membrane ion channel clock [2, 3], regulates SANC normal automaticity on a beat-to-beat basis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

The work was supported, in part, by the Intramural Research Program of the National Institute on Aging, National Institute of Health and NIH R01-HL-084487 (ALE).

Glossary

AP	action potential
DD	diastolic depolarization
LCR	local Ca^{2+} releases
LDCAE	late diastolic Ca^{2+} elevation
NCX	Na^{+} - Ca^{2+} exchanger
RyR	ryanodine receptor
SANC	sinoatrial nodal cells
SERCA	Ca^{2+} ATPase sarcoplasmic reticulum pump
SR	sarcoplasmic reticulum

References

1. Lakatta EG, Maltsev VA, Vinogradova TM. A coupled SYSTEM of intracellular Ca^{2+} clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res.* 2010; 106(4):659–73. [PubMed: 20203315]
2. Noble D, Noble PJ, Fink M. Competing oscillators in cardiac pacemaking: historical background. *Circ Res.* 2010; 106(12):1791–7. [PubMed: 20576941]
3. Himeno Y, Toyoda F, Satoh H, Amano A, Cha CY, Matsuura H, et al. Minor contribution of cytosolic Ca^{2+} transients to the pacemaker rhythm in guinea pig sinoatrial node cells. *Am J Physiol Heart Circ Physiol.* 2010; 300(1):H251–61. [PubMed: 20952667]
4. Sobie EA, Kao JP, Lederer WJ. Novel approach to real-time flash photolysis and confocal $[\text{Ca}^{2+}]$ imaging. *Pflugers Arch.* 2007; 454(4):663–73. [PubMed: 17323075]
5. Diaz ME, Trafford AW, Eisner DA. The effects of exogenous calcium buffers on the systolic calcium transient in rat ventricular myocytes. *Biophys J.* 2001; 80(4):1915–25. [PubMed: 11259304]

6. Escobar AL, Velez P, Kim AM, Cifuentes F, Fill M, Vergara JL. Kinetic properties of DM-nitrophen and calcium indicators: rapid transient response to flash photolysis. *Pflugers Arch.* 1997; 434(5):615–31. [PubMed: 9242727]
7. Momotake A, Lindegger N, Niggli E, Barsotti RJ, Ellis-Davies GC. The nitrodibenzofuran chromophore: a new caging group for ultra-efficient photolysis in living cells. *Nat Methods.* 2006; 3(1):35–40. [PubMed: 16369551]
8. Maltsev VA, Vinogradova TM, Stern MD, Lakatta EG. Letter to the editor: “Validating the requirement for beat-to-beat coupling of the Ca²⁺ clock and M clock in pacemaker cell normal automaticity”. *Am J Physiol Heart Circ Physiol.* 2011; 300(6):H2323–4. [PubMed: 21632980]

Figure 1.

(A) Confocal line-scan images of Ca^{2+} (expressed as a peak value (F) normalized to minimal fluorescence (F_0)) of a representative SANC before and following exposure to $15\mu\text{M}$ NP-EGTA (small white arrow heads marks LCRs). Histograms of (B) T_{50} of the AP-induced Ca^{2+} transient, and (C) the integral of Ca^{2+} release in the form of LCRs (detected by the Ca^{2+} indicator). (D) Prior to introduction of the Ca^{2+} buffer, rhythmic LCR periods were tightly linked to each AP occurrence; but in the presence of NP-EGTA, the LCR periods become dysrhythmic and uncoupled from the AP cycle length. Regression lines are shown with their respective equations.

Figure 2.

Photo-released caged Ca^{2+} acutely re-establishes coupling of Ca^{2+} clock and M clocks. (A) Prior to the flash, the Ca^{2+} buffer uncoupled LCRs (line scan image) from APs (brown lines) resulting in a slow, dysrhythmic AP firing rate. (B) Following the last of four 50 ms UV flashes (black arrows), large, synchronous LCRs emerge (small white arrows) and are coupled to the AP occurrence via an acute increase in DD rate, resulting in acute increase in AP firing rate (C). The concomitant acute increase of integral of Ca^{2+} release in the form of LCRs (detected by the Ca^{2+} indicator) (LDCaE) is shown in panel D. (Note that APs continued to be recorded for a number of beats beyond the Ca^{2+} recording). (E, F) The absence of LDCaE pre-flash and its appearance following flash. (G) The acute coupling of the clocks post flash induces a shift in the Ca^{2+} -membrane potential phase-plane diagram relates to AP cycles in E and F.