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Heterogeneity of calcium clock functions in dormant, dysrhythmically and rhythmically firing single pacemaker cells isolated from SA node

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ABSTRACT

Current understanding of how cardiac pacemaker cells operate is based mainly on studies in isolated single sinoatrial node cells (SANC), specifically those that rhythmically fire action potentials similar to the *in vivo* behavior of the intact sinoatrial node. However, only a small fraction of SANC exhibit rhythmic firing after isolation. Other SANC behaviors have not been studied.

Here, for the first time, we studied all single cells isolated from the sinoatrial node of the guinea pig, including traditionally studied rhythmically firing cells ('rhythmic SANC'), dysrhythmically firing cells ('dysrhythmic SANC') and cells without any apparent spontaneous firing activity ('dormant SANC'). Action potential-induced cytosolic Ca^{2+} transients and spontaneous local Ca^{2+} releases (LCRs) were measured with a 2D camera.

LCRs were present not only in rhythmically firing SANC, but also in dormant and dysrhythmic SANC. While rhythmic SANC were characterized by large LCRs synchronized in space and time towards late diastole, dysrhythmic and dormant SANC exhibited smaller LCRs that appeared stochastically and were widely distributed in time. β-adrenergic receptor (βAR) stimulation increased LCR size and synchronized LCR occurrences in all dysrhythmic and a third of dormant cells (25 of 75 cells tested). In response to βAR stimulation, these dormant SANC developed automaticity, and LCRs became coupled to spontaneous action potential-induced cytosolic Ca^{2+} transients. Conversely, dormant SANC that *did not* develop automaticity showed no significant change in average LCR characteristics. The majority of dysrhythmic cells became rhythmic in response to βAR stimulation, with the rate of action potential-induced cytosolic Ca^{2+} transients substantially increasing. In summary, isolated SANC can be broadly categorized into three major populations: dormant, dysrhythmic, and rhythmic. We interpret our results based on simulations of a numerical model of SANC operating as a coupled-clock system. On this basis, the two previously unstudied dysrhythmic and dormant cell populations have intrinsically partially or completely uncoupled clocks. Such cells can be recruited to fire rhythmically in response to βAR stimulation via increased rhythmic LCR activity and ameliorated coupling between the Ca^{2+} and membrane clocks.

Abbreviations: Sinoatrial node (the primary pacemaker of the heart), SA node; SANC, SA node cell; AP, action potential; Cell automaticity, ability of a cell to generate spontaneous, rhythmic APs; APCL, AP cycle length; SR, sarcoplasmic reticulum; AP-induced cytosolic Ca^{2+} transient, whole-cell cytosolic Ca^{2+} spike triggered by AP via L-type Ca^{2+} channel activation and its attendant Ca^{2+}-induced Ca^{2+} release from SR; LCR, local Ca^{2+}release from SR during diastolic depolarization; LCR period, the time period between the peak of the prior AP-induced cytosolic Ca^{2+} transient and subsequent LCR onset; Ca^{2+} clock, SR with Ca^{2+} pump and Ca^{2+} release channels generating rhythmic LCRs; M clock, ensemble of membrane electrogenic proteins (ion channels and ion exchangers) generating AP; Coupled clock, a contemporary concept of cardiac pacemaker cell function, i.e. Ca^{2+} clock generates diastolic rhythmic LCRs and respective Na^+/Ca^{2+} exchanger current accelerating diastolic depolarization, but M clock generates both APs and Ca^{2+} influx providing Ca^{2+} to Ca^{2+} clock; βAR, β-adrenergic receptor; Rhythmic SANC, SANC rhythmically firing AP-induced Ca^{2+} transients; Dysrhythmic SANC, SANC dysrhythmically firing AP-induced Ca^{2+} transients; Dormant SANC, SANC generating no AP-induced Ca^{2+} transients; CV, coefficient of variation (i.e. Standard Deviation/Mean)

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1. Introduction

Studies of isolated single sinoatrial (SA) node cells (SANC) have been fundamental in clarifying the cellular mechanisms of cardiac impulse initiation, which are both voltage-, time-, Na^+- and Ca^{2+}-dependent. While significant progress has been achieved in understanding how pacemaker cells operate, our knowledge is limited to only those cells that behave *in vitro* in a similar way to that observed in the SA node as a whole (i.e. those that beat rhythmically). However, only 10–30% of isolated cells contracted spontaneously in the original paper describing SANC isolation by Nakayama et al. [1]. The yield of spontaneously and rhythmically contracting cells has increased over time but has never approached 100%. Isolated single SANC that do not beat rhythmically, including those exhibiting dysrhythmic firing or an absence of firing, have never been studied.

In the present study, we addressed the issue of functional heterogeneity of single isolated SANC by examining Ca^{2+} dynamics in cells isolated from guinea pig SA node. We studied, for the first time, all phenotypes of isolated single SANC, including rhythmically firing cells ('rhythmic SANC'), dysrhythmically firing cells ('dysrhythmic SANC'), and cells without any apparent rhythmic activity ('dormant SANC').

The contemporary view on cardiac pacemaker function dictates that SANC generate action potentials (AP) via a coupled clock system, involving complex interaction between electrogenic proteins of the plasma membrane (the membrane or M clock) and the Ca^{2+} pumping and release apparatus of the sarcoplasmic reticulum (SR, i.e. the Ca^{2+} clock) [2]. The Ca^{2+} clock generates spontaneous, rhythmic diastolic local Ca^{2+} releases (LCRs), which activate inward Na^+/Ca^{2+} exchanger current (I_{NCX}), which in turn, accelerates diastolic depolarization, culminating in both an AP and the associated AP-induced cytosolic Ca^{2+} transient [3].

To study Ca^{2+} clock function in dormant, dysrhythmic, and rhythmic SANC, we recorded both AP-induced cytosolic Ca^{2+} transients and LCRs in a substantial number of cells (n = 215) using a high-resolution 2D camera. In prior studies, where AP and AP-induced cytosolic Ca^{2+} transients were measured simultaneously, we demonstrated that both measures of AP cycle length (APCL) are identical [4]. We have found that all cells, including dormant and dysrhythmic SANC, generate LCRs at baseline. β-adrenergic receptor (βAR) stimulation increased LCR size and enhanced temporal synchronization of LCR occurrences in both dormant and dysrhythmic cells. About one-third of dormant SANC developed automaticity in response to βAR stimulation, as LCRs became coupled to spontaneous AP-induced cytosolic Ca^{2+} transients. Conversely, dormant SANC that did not develop automaticity showed no significant change in average LCR characteristics. The majority of dysrhythmic cells also became rhythmic in response to βAR stimulation, with the rate of AP-induced cytosolic Ca^{2+} transients substantially increasing. Our results suggest that the enhancement and synchronization of LCRs are associated with increases in rate and rhythm of AP-induced cytosolic Ca^{2+} transients. Our numerical model simulations indicate that dysrhythmic and dormant cells have uncoupled or only partially coupled Ca^{2+} and membrane clocks, but these cells can fire rhythmically in response to βAR stimulation as the clocks become fully coupled.

2. Methods

2.1. Single cell preparation

SANC were isolated from 30 male guinea pigs in accordance with NIH guidelines for the care and use of animals, protocol # 034-LCS-

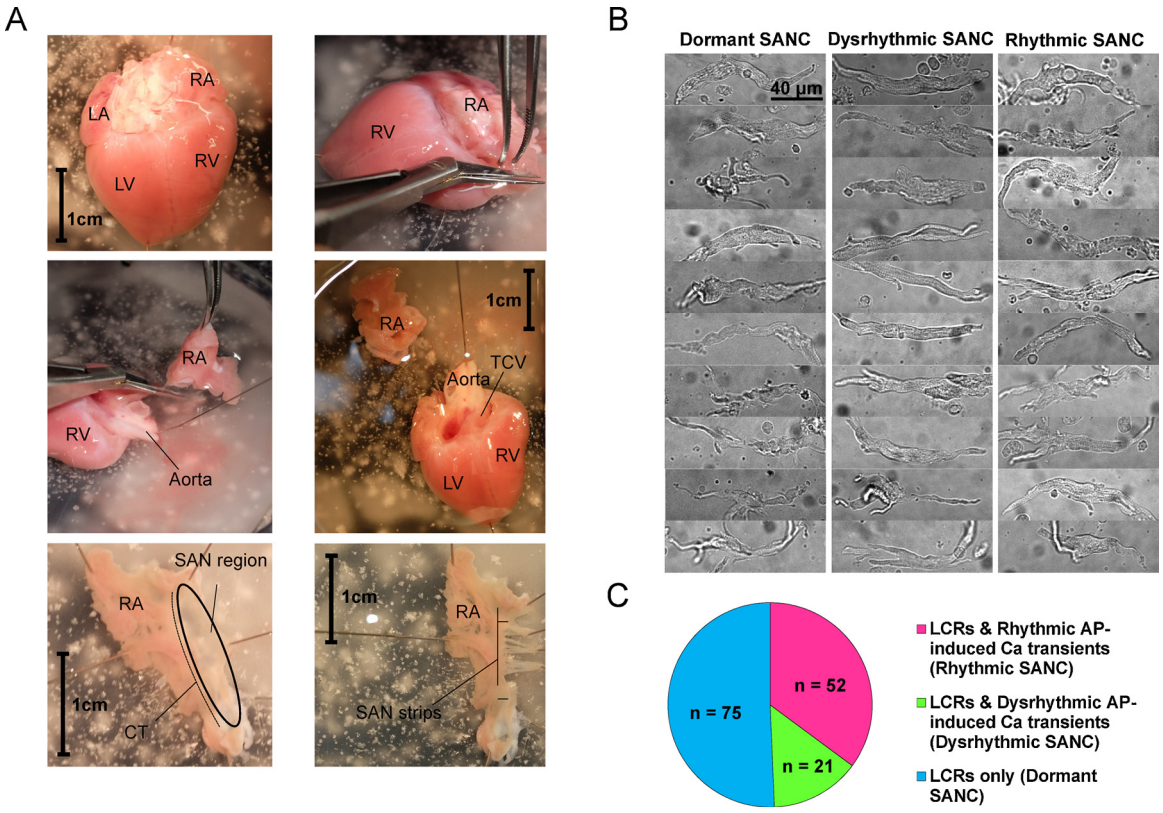

Fig. 1. Single SANC isolated from guinea pig heart exhibit a wide variety of morphologies and are heterogeneous in their intracellular Ca^{2+} dynamics at baseline. A) Location of guinea pig SA node in relation to the whole heart during our isolation procedure. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle; TCV = tricuspid valve; CT = cristae terminalis; SAN = sinoatrial node. B) Typical morphologies of the three cell populations (dormant, dysrhythmic, and rhythmic SANC). The morphologies were similar in all three cell populations. C) Population survey of single SANC with respect to their three distinct patterns of Ca^{2+} dynamics (i.e. dormant, dysrhythmic, and rhythmic SANC).