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Role of Ca^{2+} Release from Sarcoplasmic Reticulum in Pacemaker Activity of the Sinoatrial Node

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Abstract: Recent studies using confocal microscopy combined with patch clamping on single sinoatrial (SA) node pacemaker cells suggest that Ca^{2+} release from the sarcoplasmic reticulum (SR) during diastole may play a prominent role in the late phase of pacemaker depolarization. The present study was designed to test this hypothesis in the intact SA node. We investigated the effects of a high concentration of ryanodine, which is known to disable SR Ca^{2+} release, on spontaneous activity of isolated rabbit right atrium including the whole SA node by using an extracellular potential mapping technique. Inhibition of SR Ca^{2+} release by 30 μM ryanodine caused only a moderate reduction of the spontaneous firing rate (by 20.0±2.8 %, n=4) of the intact SA node. This observation is inconsistent with previous data obtained from single pacemaker cells. Physiological significance of SR Ca^{2+} release in the regulation of SA node pacemaker activity is still unsettled.

Key words: sinoatrial node, pacemaker activity, ryanodine, sarcoplasmic reticulum, extracellular potential mapping

Electrical excitation of the mammalian heart originates from specialized pacemaker cells in the sinoatrial (SA) node located in the posterior wall of the right atrium. In general, the pacemaker activity of SA node cells is thought to depend exclusively on complex interplay of many ion currents within the plasma membrane, although contribution of each ion current to pacemaker depolarization remains to be established.[1,2] However, recent studies using confocal microscopy combined with patch clamping have suggested an important role of Ca^{2+} release from the sarcoplasmic reticulum (SR) during diastole in bringing the late pacemaker potential to threshold for excitation through a stimulation of the inward Na^+/Ca^{2+} exchange current.[3-5] This hypothesis has been proposed mainly based on experimental data obtained from single SA node cells, and physiological significance of the SR Ca^{2+} release in the pacemaker activity of the intact SA node is uncertain. In the present study, we investigated the effects of a high concentration of ryanodine, which is known to disable SR Ca^{2+} release, on spontaneous activity of isolated rabbit right atrial tissue including the whole SA node.

Material and Methods

Right atrial preparations including the whole SA node and the surrounding atrial muscle were dissected from the rabbit heart as described previously,[6,7] and they were superfused with modified Krebs-Ringer solution at 32 °C. The composition of the Krebs-Ringer solution was as follows (in mM): 120.3 NaCl, 4.0 KCl, 1.2 $CaCl_2$, 1.3 $MgSO_4$, 1.2 NaH_2PO_4, 25.2 $NaHCO_3$ and 11.0 glucose (pH 7.4 when gassed with 95% O_2/5 % CO_2). Extracellular potentials recordings were made from the epicardial side of the preparations with a multiterminal grid electrode that harbored 120 pairs of modified bipolar electrodes (arranged in a 10 × 12 matrix at inter-electrode distance of 1 mm).[7,8] Electrical signals were amplified (80 dB, 0.5–30 Hz) and degitazied and stored by a personal computer equipped with an A/D board for off-line analysis. The point of initial negative deflection in each electogram was selected as the time of local activation, and isochronal lines were drawn every 5 ms. Ryanodin (Sigma, USA) was dissolved in deionized water to make a stock solution (1 mM) and an appropriate amount of stock solution was added to the perfusate to adjust a final concentration of 30 μM.

Data were expressed as mean±SEM. The statistical significance was evaluated by Student's paired *t*-test and a value of $P<0.05$ was considered significant.

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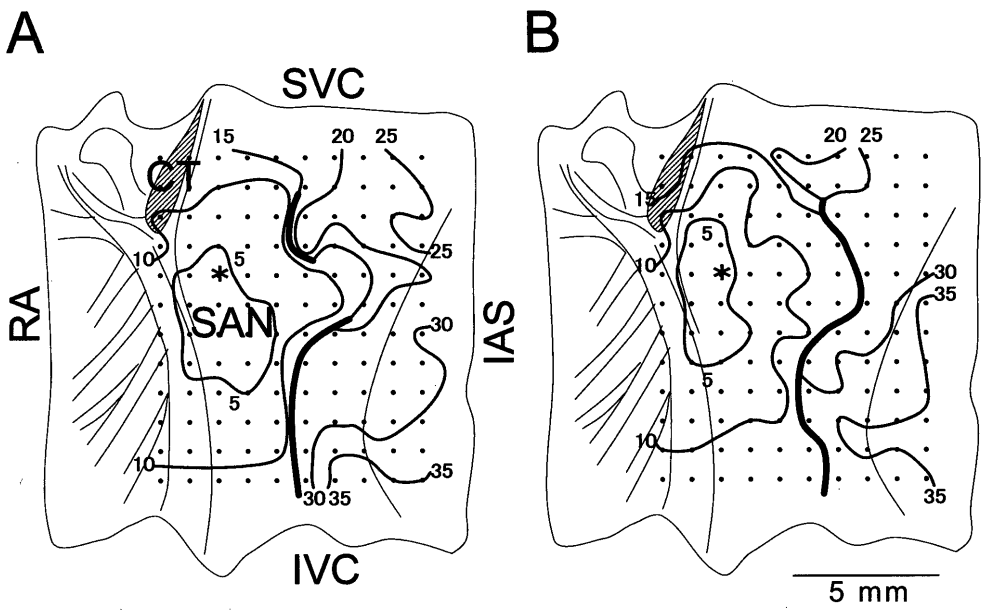

Fig. 1 Activation maps of right atrial preparation before (A) and after (B) the treatment with 30 μM ryanodien.
Small dots indicate recording sites of extracellular potentials, and thick lines are isochrones drawn every 5 ms. RA, right atrial appendage; CT, crista terminalis; SAN, sinoatial node; SVC, superior vena cava; IVC, inferior vena cava; IAS, interatrial septum: *, the position of the leading pacemaker.

Results

Under control conditions, the right atrial preparations including the SA node showed regular spontaneous activities. The average spontaneous cycle length (SCL, the time interval between successive local activation times) was 526±29 ms in four preparations. Fig. 1A illustrates a typical example of an activation map of the right atrial preparation under control condition. The site of earliest activation (leading pacemaker site) was located in the center of the SA node (* in Fig. 1A), and the excitation was propagated preferentially to the crista terminalis (CT). Isochronal crowding in the spread of excitation towards the interatial septum (IAS) indicates a zone of slow conduction around the border between the SA node and the atrial muscle in the septum. The activation sequence of spontaneous excitation showed a similar pattern in all four preparations.

The application of 30 μM ryanodien for 60 min resulted in a significant prolongation of SCL (in average by 25.2±4.1 %, n=4, $P<0.05$), but the pacemaker activity of the SA node did not cease in all the preparations studied. Fig. 1B illustrates the activation map of the same preparation shown in Fig. 1A after the treatment with 30 μM ryanodine. The site of the leading pacemaker was in the center of the SA node and the pattern of activation remained almost unchanged after the treatment with ryanodine.

Discussion

In the present study, inhibition of SR Ca^{2+} release by a high concentration of ryanodine (30 μM) caused only a moderate reduction of the spontaneous firing rate but did not stop spontaneous activity of the intact SA node tissue preparations. In single pacemaker cells isolated form the SA node, higher sensitivity of spontaneous activities to ryanodine was previously reported with IC_{50} value as low as 2.6 μM: the spontaneous activity was shown to be abolished by ryanodine at high concentrations (30–100 μM).[4,5] The large difference in the ryanodin sensitivity between single cells and the whole tissue preparations might be attributed to pronounced heterogeneity and regional difference in SA node pacemaker activity. Because action potential configurations of cells used in the previous study were compatible to those for transitional-type but not for central-type SA node pacemaker cells,[9,10] it is possible that the primary pacemaker cells in the center of the node are less sensitive to ryanodine than subsidiary pacemaker cells in the periphery. In other words, the contribution of SR Ca^{2+} release to pacemaker function could be less in the SA node primary pacemaker regions than in its periphery. We have recently reported that immunolabeling of Ca^{2+} handling proteins, including the SR Ca^{2+} release channel (ryanodine receptor), Na^+/Ca^{2+} exchanger and L-type Ca^{2+} channel, are significantly more sparse and poorly organized in the center of the rabbit SA node compared with its periphery,[11] and this may underlie the regional difference in the role of SR Ca^{2+} release.

In conclusion, physiological significance of SR Ca^{2+} release in the regulation of SA node pacemaker activity is still unsettled, and further invesigations will be required.

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