D-Ⅲ-⑤

Intracellular Calcium and Membrane Potential Oscillations in the Guinea-Pig and Rat Pulmonary Vein Myocardium

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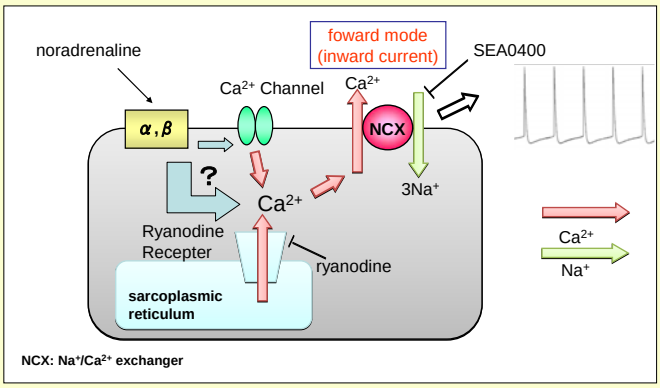
Aim

Pulmonary veins contain a myocardial layer, whose electrical activity is considered to be involved in the genesis and maintenance of atrial fibrillation.

In the present study, we applied immunohistochemical, confocal microscopic and electrophysiological analyses to isolated pulmonary veins of the guinea pig and the rat to clarify the involvement of intracellular Ca^{2+} in pulmonary vein automaticity.

Conclusion

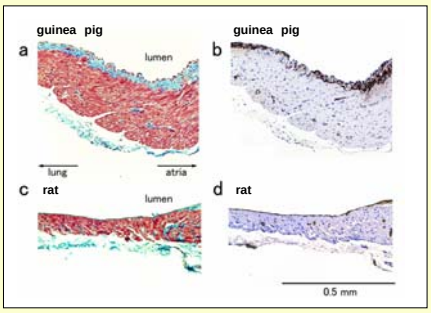
The pulmonary vein myocardium has a tendency to show spontaneous electrical activity under adrenergic influence, which is triggered by intracellular Ca^{2+} oscillations. The difference in firing pattern between the guinea-pig and rat may be due to the difference in their hyperpolarizing mechanisms.



NCX: Na^+/Ca^{2+} exchanger

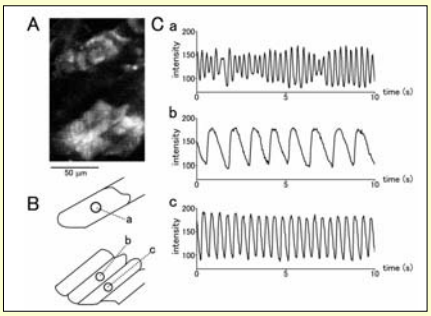
Namekata I, Tsuneoka Y, Akiba A et al., Bioimages 18, 11-22 (2010)

1. Presence of myocardial layers in the guinea-pig and rat pulmonary veins.



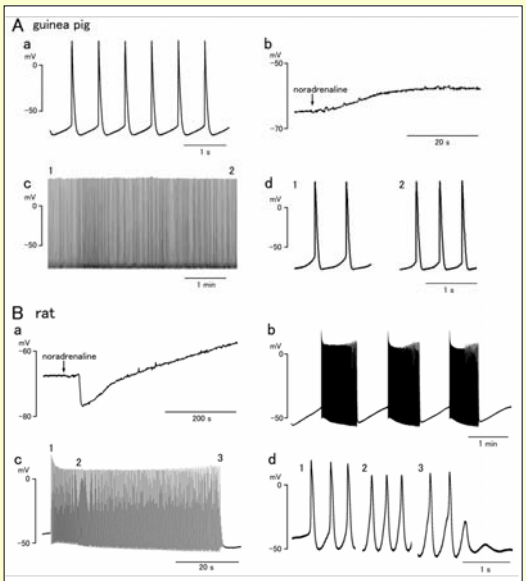
Masson-trichrome staining (a, c) and staining with anti-smooth muscle α-actin (b, d) of longitudinal sections from the guinea-pig (a, b) and rat (c, d) pulmonary veins.The pulmonary vein wall was thicker in the guinea pig than in the rat.

2. Spontaneous intracellular Ca^{2+} oscillations in pulmonary vein myocardia loaded with fluo-4.



A: Typical image of the myocardial layer in the rat. B: The three regions for quantification of Ca^{2+} fluorescence. C: Time course of the changes in fluorescence in the regions (a, b, c) indicated in B.

3. Effect of noradrenaline on the automaticity of pulmonary-vein myocardia in the guinea-pig and rat.



Aa: Spontaneous activity observed in the absence of noradrenaline. Ab: Change in resting membrane potential induced by 10 μM noradrenaline in a quiescent preparation. Ac: Noradrenaline-induced automatic activity. Ad: Waveform of the action potential observed at timepoints 1 and 2 in panel Ac. Ba: Change in resting membrane potential induced by 10 μM noradrenaline in a quiescent preparation. Bb: Noradrenaline-induced automatic activity. The first burst of this recording was shown on an expanded time scale in Bc. Bd: Waveform of the action potential observed at timepoints 1 to 3 in panel Bc.

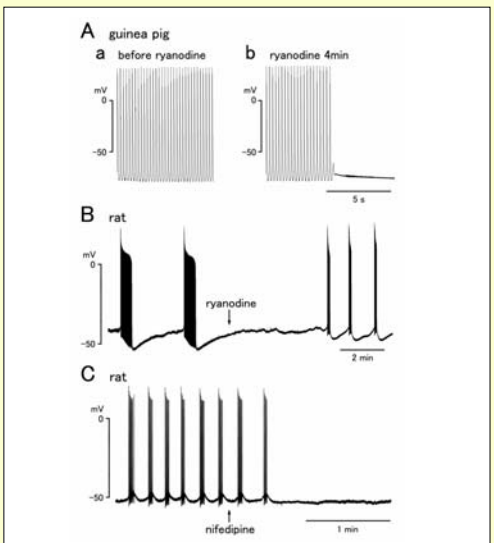
Table 1 Characteristics of automatic action potentials induced by noradrenaline in cardiac muscle of the guinea pig pulmonary vein.

	Start	1 min	5 min	10 min
maximum diastolic potential (mV)	−70.9±2.3	−72.6±2.4	−77.0±3.6	−79.3±2.2
peak (mV)	27.9±3.0	27.3±3.5	28.8±2.3	26.5±1.5
frequency (Hz)	0.4±0.1	3.1±0.3	3.1±0.4	3.4±0.4

Table 2 Characteristics of automatic action potentials and bursts of automatic activity induced by noradrenaline in cardiac muscle of the rat pulmonary vein.

	Start	Max Frequency	End
maximum diastolic potential (mV)	−60.0±2.7	−62.5±2.4	−68.4±1.9
peak (mV)	16.1±2.1	11.1±2.7	12.6±2.4
frequency (Hz)	3.4±0.4	5.3±0.5	3.6±0.5

4. Effect of ryanodine on noradrenaline-induced automatic electrical activity of pulmonary vein-myocardia in the guinea-pig and rat.

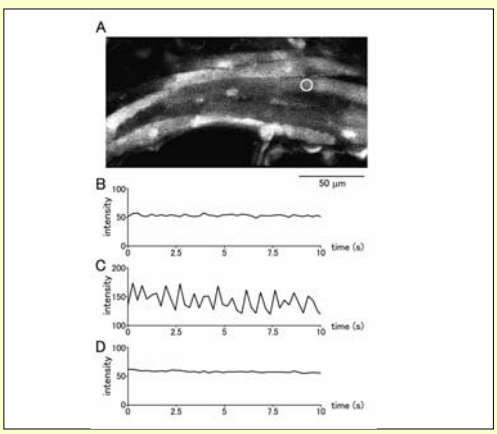


A: Automatic electrical activity before (a) and after (b) the addition of 0.1 μM ryanodine. B: Effect of 1 μM ryanodine on electrical activity. C: Effect of further application of 1 μM nifedipine to the preparation shown in panel B. All of the recordings were performed in the presence of 10 μM noradrenaline.

Table 3 Effects of ryanodine on noradrenaline induced burst electrical activity in rat pulmonary-vein myocardia.

	before ryanodine	ryanodine 5 min	ryanodine 10 min
duration (sec)	59.5±16.9	11.7±1.5*	8.7±1.3*
maximum frequency (Hz)	5.9±0.4	4.6±0.4*	3.1±0.5*

5. Noradrenaline-induced intracellular Ca^{2+} oscillations in guinea-pig pulmonary vein myocardia loaded with fluo-4.



A: Fluorescence image of the myocardial layer. The circle indicates the region of fluorescence quantification. B-D: Time course of the changes in fluorescence before addition (B) and 5 min after 10 μM noradrenaline (C) and 5 min after further application of 1 μM ryanodine (D).

Abstract

Myocardial intracellular Ca^{2+} and membrane potential oscillations were studied in the isolated guinea-pig and rat pulmonary veins with immunohistochemical, confocal microscopic and electrophysiological analyses. The myocardial layer was present between the smooth muscle layer and the adventitia, and was more developed in the guinea-pig than in the rat. Intracellular Ca^{2+} oscillations were observed in both species, which were inhibited by ryanodine. Spontaneous Ca^{2+} waves were observed to propagate along the longitudinal axis of the cell or as a spiral rotating around a subcellular core; the propagation velocity of these Ca^{2+} waves was similar to that reported in atrial and ventricular cardiomyocytes. Spontaneous action potentials were present in about 35% and 4% of the preparations from the guinea-pig and rat, respectively. In quiescent preparations from the guinea-pig, noradrenaline induced a slow depolarization of the resting membrane potential followed by constant repetitive generation of action potentials, which were inhibited by ryanodine. In quiescent preparations from the rat, noradrenaline induced an initial hyperpolarization and a subsequent depolarization of the resting membrane potential. This was followed by generation of automatic action potentials which occurred in repetitive bursts. Ryanodine either abolished or reduced the duration of action-potential bursts. These results indicate that the pulmonary vein myocardium generates automatic electrical activity under adrenergic influence, which is probably triggered by intracellular Ca^{2+} oscillations. The difference in firing pattern between the guinea-pig and rat may be due to the difference in hyperpolarizing mechanisms.

Discussion

1) The incidence of spontaneous electrical activity was higher in the guinea-pig (35%) than in the rat (3.8%), which may be partially explained by the differences in the total amount of myocardial tissue present in the pulmonary vein.

2) Noradrenaline induced changes in the resting membrane potential of quiescent preparations; depolarization in the guinea-pig (Fig. 3Ab), and a transient hyperpolarization followed by depolarization in the rat (Fig. 3Ba).
It is interesting that the maximum diastolic potential gradually shifts towards negative direction during the burst in the rat (Fig. 3B). It is likely that during the bursts, accumulation of intracellular Ca^{2+} gradually activates some Ca^{2+}-dependent hyperpolarizing currents, which eventually inhibits the generation of action potentials.

3) Result 4 indicates that both intracellular Ca^{2+}-dependent and Ca^{2+}-independent components of automatic activity exist in the rat pulmonary vein myocardium.