Topic 18 – Electrophysiology, rythmology and pacing – C

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0184

Distinct ratios of co-expressed Cx40 and Cx43 regulate a fine gap junction channel make-up and properties

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Co-expressed connexins (Cxs) can co-assemble to form gap junction channels (GJCs) of mixed Cxs composition characterized by specific electrical properties. To date the detailed GJCs make up in the distinct cardiac tissues that co-express distinct gradients of Cxs stays poorly understood. We aim to investigate whether and how the GJCs make-up depends on the level and ratios of co-expressed connexin 40 (Cx40) and Cx43. Rat Liver Epithelial cells that endogenously express Cx43 and stably transfected to induce accurate ratios Cx43:Cx40 were used to perform electrical recordings on cell pairs by applying the dual voltage clamp method. We previously showed that the induction of Cx40 decreases the electrical coupling at high ratio Cx43:Cx40 while increasing at low ratios, decreases the voltage-dependence and slows the kinetics of deactivation and of recovery of GJCs. A deeper investigation at the single channel level permits to evaluate the Cxs composition and the distribution of different kinds of GJCs. Whereas non-induced cells, are coupled by homomeric-homotypic Cx43 GJCs, induction of Cx40 modifies the GJCs population in function of the ratio Cx43:Cx40: at high ratio GJCs made of Cx43 dominate the population, whereas lower ratios favor the Cx43/Cx40 heteromerization and the contribution of the Cx40. Our data show that the GJCs make-up is finely regulated in function on the ratio Cx43:Cx40 to form functional homotypic or heteromeric GJCs with distinct contributions of Cx40 and Cx43 properties and function in regulating the impulse propagation in the healthy heart and the pro-arrhythmic Cxs dysfunction. To correlate these results with the relative cardiac Cxs expression profiles, cell pairs of different Cxs expression pattern will be studied.

0295

TRPM4 is involved in excitation-contraction coupling regulation in healthy murine atrial cardiomyocytes

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Aims: The neuropeptide Endothelin-1 (Et-1), through the inositol triphosphate (IP3) pathway, is associated with a calcium release from the sarcoplasmic reticulum (SR). This RS depletion may open Store Operated Ca^{2+} Channel (SOCs) leading to an additional calcium influx. TRPM4 is a calcium-activated non-selective cationic channel ubiquitously expressed. It is involved in many physiological processes including the negative regulation of SOCs. However, its physiological role in cardiac level remains unclear. Our purpose was to determine whether TRPM4 channel is involved during excitation-contraction coupling (ECC) at atrial level where it's normally expressed and functional, in particular in response to the Et-1 during SOCs activation.

Methods and Results: Atrial cardiomyocytes were isolated from 8 weeks-old wild-type C57bl/6J mice and ECC were analyzed by the Ionoptix® system with a myopacer and a Ca^{2+} fluorescent indicator (indo-1 AM). We used the 9-phenanthrol (10^{-5}M) (9-Phe), as a selective inhibitor of the TRPM4 channel during cardiomyocytes stimulation with Et-1 (10^{-7}M) to unmask TRPM4 function. With this approach, we demonstrated that i) as expected, Et-1 showed a positive inotropic effect on mouse atrial cardiomyocytes (sarcomere length shortening and calcium transient increased); ii) Et-1 effect was attenuated by 9-Phe application, showing an involvement of TRPM4 channel in the inotropic effect of Et-1 and iii) surprisingly the 9-Phe has an effect on calcium response without Et-1 application suggesting a role of TRPM4 channel in basal conditions.

Conclusion: This study has highlighted the involvement of TRPM4 channel in ECC of mouse atrial cardiomyocytes. In addition, Et-1 had a pro-hypertrophic effect, justifying the use of the Et-1 antagonists in humans. The TRPM4 channel, inhibited by 9-Phe, would be a possible new therapeutic target to prevent the pro-hypertrophic remodeling induced by Et-1.

0179

Specific down-regulation of cardiac connexin and pro-arrhythmic impulse propagation under electrical pacing

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The rhythmic and coordinated propagation cardiac of the action potential is ensured by gap junction channels (GJCs) that are composed of connexin40 (Cx40), Cx43, Cx45 and Cx30.2, which present specific patterns of expression and electrical properties in different functionally specialized cardiac tissues.

This study aims to determine the distinct contribution of Cxs in regulating the action potential propagation in mimicked healthy and diseased cardiac rates.

The mouse atrial phenotypic HL-1 cell line that co-expresses Cx40, Cx43 and Cx45 was seeded on microelectrodes arrays to characterize the intrinsic electrical conduction and during pacing frequency from 2 to 30Hz. Specific SiRNA transfection of each Cx is used to determine the contribution of Cxs on regulating the conduction. We previously showed that the higher the frequency, the slower the conduction velocity (CV), up to a pro-arrhythmic frequency (10Hz). After down-regulation of Cx40 (controlled by immunofluorescence), cells display a similar intrinsic coordinated activity to non-transfected cells (≈ 1Hz). However, the action potential propagates slower than non-transfected cells (≈ 60% at maximum down-regulation of Cx40). Importantly, we observed that the arrhythmic conduction occurs at lower frequency after down-regulation of Cx40.

These results indicate that Cx40 ensures a positive regulation of the action potential propagation under an increase of the cardiac rates, which suggests a specific GJCs make-up and electrical properties. Ongoing siRNA transfection for Cx43 and Cx45 will permit to distinguish the contribution of each Cxs in such regulation. Complementary biochemical characterization and patch clamp recordings on cell pairs will correlate the GJCs make-up and electrical properties to the changes of CV. This will improve our understanding on the specific role of each type of Cxs in regulating the impulse propagation in the healthy heart and their pro-arrhythmic dysfunction.

0363

Development of a cell isolation method in large mammals: regional differences in calcium signaling across left atrium from sheep

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Atrial fibrillation (AF) is the most common sustained form of arrhythmia in human. Numerous studies have showed that Ca^{2+} signaling is altered during AF, although it has not been established whether Ca^{2+} remodeling is homogenous across the different regions of the atria. Given the importance of the pulmonary veins (PV) in the treatment of AF (NEJM, 1998, 339:659-66), we have studied the characteristics of Ca^{2+} transients in 4 different regions of the left atrium (LA) in an animal model close to human.

LA myocytes were obtained by enzymatic dissociation of sheep hearts. Animals were euthanized by injection of pentobarbital and the heart was rapidly excised (guidelines approved by ethical committee). The aorta was cannulated and heart was rinsed with cardioplegic solution. The ventricles and right atrium were removed. LA was cannulated by the circumflex artery and mounted into a Langendorff perfusion system after suture of the leaky atrial branches. LA was perfused with a Ca^{2+}-free solution (~10 min), then collagenase and protease solution (0,08mM Ca^{2+}) and recirculated for ~25 min. Enzymes were washed out with a 0,2mM Ca^{2+} solution. LA was separated into

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