XXX cycle Ph.D. Course in 'Molecular Medicine'

Development and application of new methods for the detection of T-tubular morpho-functional defects in pathological isolated cardiomyocytes

2015 Annual Report

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Introduction: T-tubules are sarcolemma invaginations essential to propagate action potential (AP) during cardiac muscle contraction. T-Tubules structural remodeling has been identified in heart failure [1] and hypertrophic cardiomyopathies [2], where calcium (Ca^{2+}) homeostasis and cardiac muscle contraction are altered. However, little is known about the effects of those structural alterations on T-Tubules function.

Materials and Methods: Cardiomyocytes isolated from spontaneously hypertensive rats (SHR) were analyzed by ultrafast Random Access Two-Photon microscope (RAMP) to investigate the intracellular Ca^{2+} release during AP. Cardiomyocytes were stained with a voltage-sensitive dye and a Ca^{2+} indicator. For immunohistochemistry, fixed and permeabilized cardiomyocytes were incubated with primary and secondary antibodies against Caveolin 3, α-actinin, DHPR and RyR2.

Results: Preliminary results showed the presence of two T-Tubule populations within SHR cells: one capable of propagating AP after stimulation and the second that does not show electrical activity after stimulation. The latter population has never been observed in control cardiomyocytes (Wistar Kyoto rats, WKY). To better understand the causes of the AP propagation failure we analyzed excitation-contraction key proteins to assess whether possible alterations could be entitled for electrical defects. Cross correlation analysis between red and green fluorescence signals were performed in WKY and in SHR cells. Statistical differences were not found between the two groups. Our results show that the lack of massive structural alterations at T-Tubule and sarcomere levels may be due to T-Tubular lumen modifications that would impact on the diffusion properties of the T-tubule system. This preliminary study is a crucial point to start further investigations for understanding the mechanistic insights of the T-tubule electrical failure.

References

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Abstracts & Publications

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2015. 39th European Working Group on Cardiac Cellular Electrophysiology Meeting, Milan, Italy. "Novel insights on the relationship between T-tubular defects and contractile dysfunction in a mouse model of hypertrophic cardiomyopathy". (poster).

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